```
=> file medline hcaplus biosis biotechds embase scisearch
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY     SESSION
FULL ESTIMATED COST                                         0.21        0.21

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FILE 'SCISEARCH' ENTERED AT 10:48:55 ON 10 DEC 2004
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=> s N-acetylglucosamine-1-phosphotransferase
L1          411 N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2           184 DUP REM L1 (227 DUPLICATES REMOVED)

=> s l2 and 1975-1999/py
   3 FILES SEARCHED...
L3          146 L2 AND 1975-1999/PY

=> s human N-acetylglucosamine-1-phosphotransferase
L4            4 HUMAN N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5            4 DUP REM L4 (0 DUPLICATES REMOVED)

=> d l5 1-4 ibib ab

L5    ANSWER 1 OF 4  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-09921  BIOTECHDS
TITLE:            Novel N-acetylglucosamine-1-phosphotransferase and
                  N-acetylglucosamine-1-phosphodiester-alpha-N-
                  acetylglucosaminidase, useful for producing phosphorylated
                  lysosomal hydrolase for treating lysosomal storage diseases;
                     vector-mediated gene transfer and expression in host cell,
                     monoclonal antibody and hybridoma
AUTHOR:           Canfield W M
PATENT ASSIGNEE:  Canfield W M
LOCATION:         Oklahoma City, OK, USA.
PATENT INFO:      WO 2001019955 22 Mar 2001
APPLICATION INFO: WO 2000-US21970 14 Sep 2000
PRIORITY INFO:    US 1999-153831 14 Sep 1999
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2001-290356 [30]
AB    Isolated human N-acetylglucosamine-
      1-phosphotransferase (GlcNAc-phosphotransferase) and
      N-acetylglucosamine-1-phosphodiester-alpha-N-acetylglucosaminidase
      (phosphodiester-alpha-GlcNAcase, EC-3.1.4.45), is new.  Also claimed are:
```

nucleic acids encoding GlcNAc-phosphotransferase and phosphodiester alpha-GlcNAcase; vector containing the nucleic acids; host cell containing the vector; preparation of GlcNAc-phosphotransferase or phosphodiester-alpha-GlcNAcase; nucleic acids encoding mouse GlcNAc-phosphotransferase which has an alpha-subunit, beta-subunit and gamma-subunit and mouse phosphodiester-alpha-GlcNAcase; vector and host cell transformed with this vector; preparation of mouse GlcNAc-phosphotransferase and phosphodiester-alpha-GlcNAcase; lysosomal hydrolase containing a mannose-6-phosphate; phosphorylated lysosomal hydrolase; producing a high mannose lysosomal hydrolase; high mannose lysosomal hydrolase; and monoclonal antibodies produced by PT18 hybridoma (ATCC PTA 2432) or UC1 hybridoma (ATCC 2431). The GlcNAc-phosphotransferase and phosphodiester-alpha-GlcNAcase are useful for producing a phosphorylated lysosomal hydrolase for treating lysosomal storage disease. (91pp)

L5 ANSWER 2 OF 4 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1992:112469 BIOSIS
DOCUMENT NUMBER: PREV199242052469; BR42:52469
TITLE: BIOCHEMICAL HETEROGENEITY AND PRENATAL DIAGNOSIS OF MUCOLIPIDOSES II AND III.
AUTHOR(S): BEN-YOSEPH Y [Reprint author]; MITCHELL D A; YAGER R M
CORPORATE SOURCE: WAYNE STATE UNIVERSITY SCHOOL MED, DETROIT, MI, USA
SOURCE: American Journal of Human Genetics, (1991) Vol. 49, No. 4 SUPPL, pp. 94.
Meeting Info.: PROCEEDINGS OF THE 8TH INTERNATIONAL CONGRESS OF HUMAN GENETICS, WASHINGTON, D.C., USA, OCTOBER 6-11, 1991. AM J HUM GENET.
CODEN: AJHGAG. ISSN: 0002-9297.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 24 Feb 1992
Last Updated on STN: 24 Feb 1992

L5 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1990:40514 BIOSIS
DOCUMENT NUMBER: PREV199038019744; BR38:19744
TITLE: CORRECTION OF CLASSICAL MUCOLIPIDOSIS III BY GENE TRANSFER.
AUTHOR(S): FOWLER M L [Reprint author]; SHOWS T B
CORPORATE SOURCE: ROSWELL PARK MEML INST, BUFFALO, NY, USA
SOURCE: American Journal of Human Genetics, (1989) Vol. 45, No. 4 SUPPL, pp. A5.
Meeting Info.: 40TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS, BALTIMORE, MARYLAND, USA, NOVEMBER 11-15, 1989. AM J HUM GENET.
CODEN: AJHGAG. ISSN: 0002-9297.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 28 Dec 1989
Last Updated on STN: 1 Feb 1990

L5 ANSWER 4 OF 4 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1985:150907 BIOSIS
DOCUMENT NUMBER: PREV198529040903; BR29:40903
TITLE: A MODEL SYSTEM TO STUDY **HUMAN N ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE.**
AUTHOR(S): LITTLE L E [Reprint author]; ALCOULOMBRE M; DROTAR A M; MILLER A L
CORPORATE SOURCE: UNIV CALIF, SAN DIEGO, CALIF 92093, USA
SOURCE: Federation Proceedings, (1985) Vol. 44, No. 5, pp. 1408.
Meeting Info.: 69TH ANNUAL MEETING OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY, ANAHEIM,

CALIF., USA, APR. 21-26, 1985. FED PROC.
CODEN: FEPRA7. ISSN: 0014-9446.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

=> s (human N-acetylglucosamine-1-phosphotransferase or human GlcNAc-phosphotransferase)
L6 6 (HUMAN N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE OR HUMAN GLCNAC-PHOSPHOTRANSFERASE)

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7 6 DUP REM L6 (0 DUPLICATES REMOVED)

=> d l7 1-6 ibib ab

L7 ANSWER 1 OF 6 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2003:513487 BIOSIS
DOCUMENT NUMBER: PREV200300516878
TITLE: Unfortunate oversight.
AUTHOR(S): Leroy, Jules G. [Reprint Author]
CORPORATE SOURCE: Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA
SOURCE: American Journal of Medical Genetics, (September 15 2003) Vol. 122A, No. 1, pp. 92-93. print.
ISSN: 0148-7299 (ISSN print).
DOCUMENT TYPE: Letter
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Nov 2003
Last Updated on STN: 5 Nov 2003

L7 ANSWER 2 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:550993 HCAPLUS
DOCUMENT NUMBER: 139:112730
TITLE: Method for production of highly phosphorylated human acid .beta.-glucocerebrosidase (GBA), and use of GBA in treating bone or lung tissue of patient with Gaucher's disease
INVENTOR(S): Canfield, William
PATENT ASSIGNEE(S): Novazyme Pharmaceuticals, Inc., USA
SOURCE: U.S. Pat. Appl. Publ., 54 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003133924	A1	20030717	US 2001-24197	20011221
PRIORITY APPLN. INFO.:			US 2001-24197	20011221

AB The invention provides a method for producing a highly phosphorylated acid .beta.-glucocerebrosidase (GBA), which involves: (a) culturing cells transfected with polynucleotides encoding a recombinant GBA in the presence of at least one .alpha.1,2-mannosidase inhibitor; (b) recovering high mannose recombinant GBA from said cells; (c) contacting said GBA with an isolated N-acetylglucosaminyl phosphotransferase (GlcNAc phosphotransferase) to produce a modified GBA; and (d) contacting said modified GBA with N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase (phosphodiester .alpha.-GlcNAcase). The invention also provides for the use of said highly phosphorylated GBA in treating bone or lung tissue of a patient suffering from Gaucher's disease. The invention further provides the cDNA and amino acid sequences of human GBA, phosphodiester .alpha.-GlcNAcase, and GlcNAc

phosphotransferase. The invention relates that said GlcNAc phosphotransferase comprises an .alpha. and .beta. subunit, which reduces substrate specificity, and allows the GlcNAc phosphotransferase to catalyze the transfer of N-acetylglucosamine-1-phosphate from UDP-GlcNAc to the GBA enzyme. The invention discussed that in this method GBA will be phosphorylated which will allow binding to mannose 6 receptors on the surface of lung and bone cells. In so binding to the receptor on these tissues, the problems of the current GBA replacement therapy can be addressed.

L7 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-09921 BIOTECHDS
TITLE: Novel N-acetylglucosamine-1-phosphotransferase and N-acetylglucosamine-1-phosphodiester-alpha-N-acetylglucosaminidase, useful for producing phosphorylated lysosomal hydrolase for treating lysosomal storage diseases; vector-mediated gene transfer and expression in host cell, monoclonal antibody and hybridoma
AUTHOR: Canfield W M
PATENT ASSIGNEE: Canfield W M
LOCATION: Oklahoma City, OK, USA.
PATENT INFO: WO 2001019955 22 Mar 2001
APPLICATION INFO: WO 2000-US21970 14 Sep 2000
PRIORITY INFO: US 1999-153831 14 Sep 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-290356 [30]
AB Isolated **human N-acetylglucosamine-1-phosphotransferase** (GlcNAc-phosphotransferase) and N-acetylglucosamine-1-phosphodiester-alpha-N-acetylglucosaminidase (phosphodiester-alpha-GlcNAcase, EC-3.1.4.45), is new. Also claimed are: nucleic acids encoding GlcNAc-phosphotransferase and phosphodiester alpha-GlcNAcase; vector containing the nucleic acids; host cell containing the vector; preparation of GlcNAc-phosphotransferase or phosphodiester-alpha-GlcNAcase; nucleic acids encoding mouse GlcNAc-phosphotransferase which has an alpha-subunit, beta-subunit and gamma-subunit and mouse phosphodiester-alpha-GlcNAcase; vector and host cell transformed with this vector; preparation of mouse GlcNAc-phosphotransferase and phosphodiester-alpha-GlcNAcase; lysosomal hydrolase containing a mannose-6-phosphate; phosphorylated lysosomal hydrolase; producing a high mannose lysosomal hydrolase; high mannose lysosomal hydrolase; and monoclonal antibodies produced by PT18 hybridoma (ATCC PTA 2432) or UC1 hybridoma (ATCC 2431). The GlcNAc-phosphotransferase and phosphodiester-alpha-GlcNAcase are useful for producing a phosphorylated lysosomal hydrolase for treating lysosomal storage disease. (91pp)

L7 ANSWER 4 OF 6 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1992:112469 BIOSIS
DOCUMENT NUMBER: PREV199242052469; BR42:52469
TITLE: BIOCHEMICAL HETEROGENEITY AND PRENATAL DIAGNOSIS OF MUCOLIPIDOSES II AND III.
AUTHOR(S): BEN-YOSEPH Y [Reprint author]; MITCHELL D A; YAGER R M
CORPORATE SOURCE: WAYNE STATE UNIVERSITY SCHOOL MED, DETROIT, MI, USA
SOURCE: American Journal of Human Genetics, (1991) Vol. 49, No. 4 SUPPL, pp. 94.
Meeting Info.: PROCEEDINGS OF THE 8TH INTERNATIONAL CONGRESS OF HUMAN GENETICS, WASHINGTON, D.C., USA, OCTOBER 6-11, 1991. AM J HUM GENET.
CODEN: AJHGAG. ISSN: 0002-9297.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 24 Feb 1992
Last Updated on STN: 24 Feb 1992

L7 ANSWER 5 OF 6 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1990:40514 BIOSIS
DOCUMENT NUMBER: PREV199038019744; BR38:19744
TITLE: CORRECTION OF CLASSICAL MUCOLIPIDOSIS III BY GENE TRANSFER.
AUTHOR(S): FOWLER M L [Reprint author]; SHOWS T B
CORPORATE SOURCE: ROSWELL PARK MEML INST, BUFFALO, NY, USA
SOURCE: American Journal of Human Genetics, (1989) Vol. 45, No. 4 SUPPL, pp. A5.
Meeting Info.: 40TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS, BALTIMORE, MARYLAND, USA, NOVEMBER 11-15, 1989. AM J HUM GENET.
CODEN: AJHGAG. ISSN: 0002-9297.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 28 Dec 1989
Last Updated on STN: 1 Feb 1990

L7 ANSWER 6 OF 6 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1985:150907 BIOSIS
DOCUMENT NUMBER: PREV198529040903; BR29:40903
TITLE: A MODEL SYSTEM TO STUDY **HUMAN N ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE.**
AUTHOR(S): LITTLE L E [Reprint author]; ALCOULOMBRE M; DROTAR A M; MILLER A L
CORPORATE SOURCE: UNIV CALIF, SAN DIEGO, CALIF 92093, USA
SOURCE: Federation Proceedings, (1985) Vol. 44, No. 5, pp. 1408.
Meeting Info.: 69TH ANNUAL MEETING OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY, ANAHEIM, CALIF., USA, APR. 21-26, 1985. FED PROC.
CODEN: FEPRA7. ISSN: 0014-9446.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

=> FIL STNGUIDE

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	28.92	29.13

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-0.70	-0.70

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LAST RELOADED: Dec 3, 2004 (20041203/UP).

=> s registry
L8 105 REGISTRY

=> file registry

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FULL ESTIMATED COST	0.18	29.31

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION

CA SUBSCRIBER PRICE 0.00 -0.70

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DICTIONARY FILE UPDATES: 8 DEC 2004 HIGHEST RN 795251-52-4

TSCA INFORMATION NOW CURRENT THROUGH MAY 21, 2004

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Experimental and calculated property data are now available. For more
information enter HELP PROP at an arrow prompt in the file or refer
to the file summary sheet on the web at:
http://www.cas.org/ONLINE/DBSS/registryss.html

```
=> s human N-acetylglucosamine-      1-phosphotransferase
       4343814 HUMAN
       5734730 N
          2357 ACETYLGLUCOSAMINE
      16377278 1
          1668 PHOSPHOTRANSFERASE
L9           0 HUMAN N-ACETYLGLUCOSAMINE-      1-PHOSPHOTRANSFERASE
               (HUMAN(W)N(W)ACETYLGLUCOSAMINE(W)1(W)PHOSPHOTRANSFERASE)

=> s  N-acetylglucosamine-1-phosphotransferase
       5734730 N
          2357 ACETYLGLUCOSAMINE
      16377278 1
          1668 PHOSPHOTRANSFERASE
L10          3 N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
               (N(W)ACETYLGLUCOSAMINE(W)1(W)PHOSPHOTRANSFERASE)

=> d l10 1-3

L10  ANSWER 1 OF 3  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   163649-60-3  REGISTRY
CN   Acetylglucosamine-1-phosphotransferase, uridine diphosphoacetylglucosamine-
     protein (serine) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Protein (serine) N-acetylglucosamine-1-phosphotransferase
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS
DT.CA  CAplus document type:  Journal
RL.NP  Roles from non-patents:  PREP (Preparation); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L10  ANSWER 2 OF 3  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   84012-69-1  REGISTRY
CN   Acetylglucosamine-1-phosphotransferase, uridine diphosphoacetylglucosamine-
     lysosomal enzyme precursor (9CI)  (CA INDEX NAME)
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OTHER NAMES:
CN Acetylglucosamine-1-phosphotransferase, uridine diphosphoacetylglucosamine-glycoprotein
CN E.C. 2.7.8.17
CN Lysosomal enzyme acetylglucosamine-1-phosphotransferase
CN **Lysosomal enzyme N-acetylglucosamine-1-phosphotransferase**
CN Lysosomal enzyme precursor acetylglucosamine-1-phosphotransferase
CN N-Acetylglucosaminyl phosphotransferase
CN UDP-acetylglucosamine:lysosomal enzyme precursor acetylglucosamine-1-phosphotransferase
CN **UDP-GlcNAc:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase**
CN **UDP-N-acetylglucosamine:glycoprotein N-acetylglucosamine-1-phosphotransferase**
CN UDP-N-acetylglucosamine:glycoprotein N-acetylglucosaminyl-1-phosphotransferase
DR 78169-43-4
MF Unspecified
CI MAN
LC STN Files: BIOSIS, CA, CAPLUS, TOXCENTER, USPAT2, USPATFULL
DT.CA CAplus document type: Conference; Dissertation; Journal; Patent
RL.P Roles from patents: BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); PRP (Properties); USES (Uses)
RL.NP Roles from non-patents: ANST (Analytical study); BIOL (Biological study); FORM (Formation, nonpreparative); OCCU (Occurrence); PREP (Preparation); PROC (Process); PRP (Properties); USES (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
87 REFERENCES IN FILE CA (1907 TO DATE)
87 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L10 ANSWER 3 OF 3 REGISTRY COPYRIGHT 2004 ACS on STN
RN 70431-08-2 REGISTRY
CN Acetylglucosamine-1-phosphotransferase, uridine diphosphoacetylglucosamine-dolichyl phosphate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Chitobiosylpyrophosphoryldolichol synthase
CN Dolichol phosphate acetylglucosamine-1-phosphotransferase
CN **Dolichol phosphate N-acetylglucosamine-1-phosphotransferase**
CN **Dolichyl phosphate N-acetylglucosamine-1-phosphotransferase**
CN E.C. 2.7.8.15
CN N-Acetylglucosaminylpyrophosphoryldolichol synthase
CN UDP-acetylglucosamine-dolichol phosphate acetylglucosamine phosphotransferase
CN UDP-acetylglucosamine-dolichol phosphate acetylglucosamine-1-phosphotransferase
CN UDP-N-Acetylglucosamine:dolichyl phosphate N-acetylglucosaminyl 1-phosphotransferase
CN Uridine diphosphate N-acetylglucosamine:dolichol phosphate N-acetylglucosamine phosphate transferase
MF Unspecified
CI MAN
LC STN Files: BIOSIS, CA, CAPLUS, TOXCENTER, USPAT2, USPATFULL
DT.CA CAplus document type: Conference; Dissertation; Journal; Patent
RL.P Roles from patents: ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
RL.NP Roles from non-patents: BIOL (Biological study); FORM (Formation, nonpreparative); OCCU (Occurrence); PREP (Preparation); PROC (Process); PRP (Properties); RACT (Reactant or reagent); USES (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
125 REFERENCES IN FILE CA (1907 TO DATE)
125 REFERENCES IN FILE CAPLUS (1907 TO DATE)

```
=> s GlcNAc-phosphotransferase
           211 GLCNAC
          1668 PHOSPHOTRANSFERASE
L11          5 GLCNAC-PHOSPHOTRANSFERASE
                 (GLCNAC(W)PHOSPHOTRANSFERASE)

=> d l11 1-5

L11  ANSWER 1 OF 5  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   562882-89-7  REGISTRY
CN   Acetylglucosamine-1-phosphotransferase, uridine diphosphoacetylglucosamine-
     lysosomal enzyme precursor (human subunit .beta.) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   5: PN: US20030133924 SEQID: 5 claimed protein
CN   GlcNAc phosphotransferase (human subunit .beta.)
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
DT.CA  CAplus document type:  Patent
RL.P   Roles from patents:  BIOL (Biological study); PRP (Properties); USES
       (Uses)

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L11  ANSWER 2 OF 5  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   562882-88-6  REGISTRY
CN   Acetylglucosamine-1-phosphotransferase, uridine diphosphoacetylglucosamine-
     lysosomal enzyme precursor (human subunit .alpha.) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   4: PN: US20030133924 SEQID: 4 claimed protein
CN   GlcNAc phosphotransferase (human subunit .alpha.)
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
DT.CA  CAplus document type:  Patent
RL.P   Roles from patents:  BIOL (Biological study); PRP (Properties); USES
       (Uses)

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L11  ANSWER 3 OF 5  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   562882-87-5  REGISTRY
CN   DNA (human uridine diphosphoacetylglucosamine-lysosomal enzyme precursor
     acetylglucosamine-1-phosphotransferase cDNA plus flanks) (9CI)  (CA INDEX
     NAME)
OTHER NAMES:
CN   3: PN: US20030133924 SEQID: 3 claimed DNA
CN   DNA (human GlcNAc phosphotransferase cDNA plus flanks)
FS   NUCLEIC ACID SEQUENCE
MF   Unspecified
CI   MAN
```

SR CA
LC STN Files: CA, CAPLUS, USPATFULL
DT.CA CAplus document type: Patent
RL.P Roles from patents: BIOL (Biological study); PRP (Properties)

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L11 ANSWER 4 OF 5 REGISTRY COPYRIGHT 2004 ACS on STN
RN 562882-86-4 REGISTRY
CN Acetylglucosamine-1-phosphotransferase, uridine diphosphoacetylglucosamine-lysosomal enzyme precursor (human subunit .alpha.) fusion protein with proteolytic cleavage site peptide (synthetic) fusion protein with uridine diphosphoacetylglucosamine-lysosomal enzyme precursor acetylglucosamine-1-phosphotransferase (human subunit .beta.) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 2: PN: US20030133924 SEQID: 2 claimed protein
CN **Soluble GlcNAc phosphotransferase (synthetic human)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, USPATFULL
DT.CA CAplus document type: Patent
RL.P Roles from patents: BIOL (Biological study); PRP (Properties); USES (Uses)

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L11 ANSWER 5 OF 5 REGISTRY COPYRIGHT 2004 ACS on STN
RN 562882-85-3 REGISTRY
CN DNA (human uridine diphosphoacetylglucosamine-lysosomal enzyme precursor acetylglucosamine-1-phosphotransferase subunit .alpha. fusion protein with synthetic proteolytic cleavage site peptide fusion protein with uridine diphosphoacetylglucosamine-lysosomal enzyme precursor acetylglucosamine-1-phosphotransferase subunit .beta.-specifying cDNA) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 1: PN: US20030133924 SEQID: 1 claimed DNA
CN **DNA (human soluble GlcNAc phosphotransferase-specifying cDNA)**
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, USPATFULL
DT.CA CAplus document type: Patent
RL.P Roles from patents: BIOL (Biological study); PRP (Properties)

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> d his

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     (FILE 'HOME' ENTERED AT 10:48:21 ON 10 DEC 2004)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, EMBASE, SCISEARCH' ENTERED AT
     10:48:55 ON 10 DEC 2004
L1           411 S N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L2           184 DUP REM L1 (227 DUPLICATES REMOVED)
L3           146 S L2 AND 1975-1999/PY
L4             4 S HUMAN N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L5             4 DUP REM L4 (0 DUPLICATES REMOVED)
L6             6 S (HUMAN N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE OR HUMAN GLCN
L7             6 DUP REM L6 (0 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 10:54:39 ON 10 DEC 2004
L8           105 S REGISTRY

     FILE 'REGISTRY' ENTERED AT 10:56:18 ON 10 DEC 2004
L9             0 S HUMAN N-ACETYLGLUCOSAMINE-      1-PHOSPHOTRANSFERASE
L10            3 S  N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L11            5 S GLCNAC-PHOSPHOTRANSFERASE

=> s  N-acetylglucosamine-1-phosphodiester N-acetylglucosaminidase
       5734730 N
          2357 ACETYLGLUCOSAMINE
      16377278 1
          1893 PHOSPHODIESTER
       5734730 N
           163 ACETYLGLUCOSAMINIDASE
L12            0 N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER N-ACETYLGLUCOSAMINIDASE
                 (N(W)ACETYLGLUCOSAMINE(W)1(W)PHOSPHODIESTER(W)N(W)ACETYLGLUCOS
                 AMINIDASE)

=> s phosphodiester-GlcNAase
          1893 PHOSPHODIESTER
             0 GLCNAASE
L13            0 PHOSPHODIESTER-GLCNAASE
                 (PHOSPHODIESTER(W)GLCNAASE)

=> s phosphodiester-GlcNAcase
          1893 PHOSPHODIESTER
             2 GLCNACASE
L14            0 PHOSPHODIESTER-GLCNACASE
                 (PHOSPHODIESTER(W)GLCNACASE)

=> s phosphodiester-GlcNAcase
          1893 PHOSPHODIESTER
             2 GLCNACASE
L15            0 PHOSPHODIESTER-GLCNACASE
                 (PHOSPHODIESTER(W)GLCNACASE)

=> d his

     (FILE 'HOME' ENTERED AT 10:48:21 ON 10 DEC 2004)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, EMBASE, SCISEARCH' ENTERED AT
     10:48:55 ON 10 DEC 2004
L1           411 S N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L2           184 DUP REM L1 (227 DUPLICATES REMOVED)
L3           146 S L2 AND 1975-1999/PY
L4             4 S HUMAN N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L5             4 DUP REM L4 (0 DUPLICATES REMOVED)
L6             6 S (HUMAN N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE OR HUMAN GLCN
L7             6 DUP REM L6 (0 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 10:54:39 ON 10 DEC 2004
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```
L8          105 S REGISTRY

     FILE 'REGISTRY' ENTERED AT 10:56:18 ON 10 DEC 2004
L9            0 S HUMAN N-ACETYLGLUCOSAMINE-      1-PHOSPHOTRANSFERASE
L10           3 S  N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L11           5 S GLCNAC-PHOSPHOTRANSFERASE
L12           0 S  N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER N-ACETYLGLUCOSAMINIDASE
L13           0 S PHOSPHODIESTER-GLCNAASE
L14           0 S PHOSPHODIESTER-GLCNACASE
L15           0 S PHOSPHODIESTER-GLCNACASE

=> file hcaplus
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                     122.35     151.66

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                         ENTRY    SESSION
CA SUBSCRIBER PRICE                                       0.00      -0.70

FILE 'HCAPLUS' ENTERED AT 11:05:26 ON 10 DEC 2004
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FILE COVERS 1907 - 10 Dec 2004  VOL 141 ISS 25
FILE LAST UPDATED: 9 Dec 2004  (20041209/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.


=> s N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER N-ACETYLGLUCOSAMINIDASE
       2752725 N
          11396 ACETYLGLUCOSAMINE
       8111600 1
          5587 PHOSPHODIESTER
       2752725 N
          4080 ACETYLGLUCOSAMINIDASE
L16           2 N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER N-ACETYLGLUCOSAMINIDASE
                (N(W)ACETYLGLUCOSAMINE(W)1(W)PHOSPHODIESTER(W)N(W)ACETYLGLUCOS
                AMINIDASE)

=> dup rem l16
PROCESSING COMPLETED FOR L16
L17           2 DUP REM L16 (0 DUPLICATES REMOVED)

=> d l17 1-2 ibib ab

L17  ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2003:492554  HCAPLUS
DOCUMENT NUMBER:          139:65404
TITLE:                    Soluble human acetylglucosamine-1-phosphotransferase
                          containing an artificial proteolytic cleavage site to
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generate .alpha. and .beta. subunits
INVENTOR(S): Canfield, William; Kudo, Mariko
PATENT ASSIGNEE(S): Novazyme Pharmaceuticals, Inc., USA
SOURCE: U.S. Pat. Appl. Publ., 55 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003119088	A1	20030626	US 2001-23888	20011221
WO 2003057826	A2	20030717	WO 2002-US37624	20021220

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-23888 A 20011221

AB Recombinant sol. UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (I, EC 2.7.8.17) is not efficiently subject to post-translational proteolytic cleavage when expressed in mammalian cells and uncleaved forms have poor GlcNAc phosphotransferase activity. To solve this problem, the invention shows that by interposing a unique proteolytic cleavage site between the .alpha. and .beta. subunits in the I polyprotein, the polyprotein is cleaved and when expressed with the .gamma. subunit, effectively phosphorylates an enzyme substrate. In addn., the .alpha. and .beta. subunits alone without the .gamma. subunit are catalytically active. Furthermore, the absence of the .gamma. subunit results in loss of substrate specificity to only those lysosomal enzymes targeted via the mannose-6-phosphate targeting systems, e.g., acid .alpha.-glucosidase, acid .beta.-galactosidase, .beta.-hexaminidase, and others. This loss of substrate specificity allows the sol. I contg. the .alpha. and .beta. tetramer to effectively phosphorylate any glycoprotein having an appropriate acceptor oligosaccharide. Patients suffering from a lysosomal storage disease can be treated by contacting a lysosomal hydrolase with sol. I to produce a lysosomal hydrolase with an N-acetylglucosamine-1-phosphate, removing the N-acetylglucosamine by contact of the lysosomal hydrolase with a **N-acetylglucosamine-1-phosphodiester-N-acetylglucosaminidase** (EC 3.1.4.45) to produce a phosphorylated lysosomal hydrolase, and administering an amt. of the phosphorylated enzyme sufficient to treat said disease.

L17 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1990:526117 HCAPLUS
DOCUMENT NUMBER: 113:126117
TITLE: Brefeldin A prevents uncovering but not phosphorylation of the recognition marker in cathepsin D
AUTHOR(S): Radons, Juergen; Isidoro, Ciro; Hasilik, Andrej
CORPORATE SOURCE: Inst. Physiol. Chem. Pathobiochem., Westfaelische Wilhelms-Univ., Muenster, D-4400, Germany
SOURCE: Biological Chemistry Hoppe-Seyler (1990), 371(7), 567-73
CODEN: BCHSEI; ISSN: 0177-3593
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Brefeldin A (BFA) has been shown to inhibit transiently the subcellular

transport of cathepsin D. The effect of this antibiotic on processing of the phosphorylated oligosaccharides in cathepsin D was studied in human promonocytes U937. In the presence of the drug the phosphorylation of cathepsin D precursor continued at a diminished rate. The phosphorylated oligosaccharides in cathepsin D comprised mono- and bis-phosphorylated forms. The relative amts. of the two species were not changed in the presence of BFA. The uncovering of the phosphate groups and the proteolytic processing of the phosphorylated precursor were abolished. In an in vitro assay the uncovering enzyme, **N-acetylglucosamine-1-phosphodiester N-acetylglucosaminidase** was not inhibited by BFA. Thus, this drug interrupts the traffic between the compartments contg. N-acetylglucosaminyl phosphotransferase and **N-acetylglucosamine-1-phosphodiester N-acetylglucosaminidase**.

```
=> s phosphodiester GlcNAcase
          5587 PHOSPHODIESTER
            63 GLCNACASE
L18          0 PHOSPHODIESTER GLCNACASE
                 (PHOSPHODIESTER(W)GLCNACASE)

=> s N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER alpha- N-ACETYLGLUCOSAMINIDASE
       2752725 N
         11396 ACETYLGLUCOSAMINE
       8111600 1
          5587 PHOSPHODIESTER
       1512236 ALPHA
       2752725 N
          4080 ACETYLGLUCOSAMINIDASE
L19         22 N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER ALPHA- N-ACETYLGLUCOSAMINID
               ASE
                 (N(W)ACETYLGLUCOSAMINE(W)1(W)PHOSPHODIESTER(W)ALPHA(W)N(W)ACET
                 YLGLUCOSAMINIDASE)

=> dup rem l19
PROCESSING COMPLETED FOR L19
L20          22 DUP REM L19 (0 DUPLICATES REMOVED)

=> d l20 1-22 ibib ab

L20  ANSWER 1 OF 22  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2003:550993  HCAPLUS
DOCUMENT NUMBER:          139:112730
TITLE:                    Method for production of highly phosphorylated human
                          acid .beta.-glucocerebrosidase (GBA), and use of GBA
                          in treating bone or lung tissue of patient with
                          Gaucher's disease
INVENTOR(S):              Canfield, William
PATENT ASSIGNEE(S):       Novazyme Pharmaceuticals, Inc., USA
SOURCE:                   U.S. Pat. Appl. Publ., 54 pp.
                          CODEN: USXXCO
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
     ---------------      ----   --------    --------------------    --------
     US 2003133924         A1    20030717    US 2001-24197           20011221
PRIORITY APPLN. INFO.:                       US 2001-24197           20011221
AB   The invention provides a method for producing a highly phosphorylated acid
     .beta.-glucocerebrosidase (GBA), which involves: (a) culturing cells
     transfected with polynucleotides encoding a recombinant GBA in the
```

presence of at least one .alpha.1,2-mannosidase inhibitor; (b) recovering high mannose recombinant GBA from said cells; (c) contacting said GBA with an isolated N-acetylglucosaminyl phosphotransferase (GlcNAc phosphotransferase) to produce a modified GBA; and (d) contacting said modified GBA with **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (phosphodiester .alpha.-GlcNAcase). The invention also provides for the use of said highly phosphorylated GBA in treating bone or lung tissue of a patient suffering from Gaucher's disease. The invention further provides the cDNA and amino acid sequences of human GBA, phosphodiester .alpha.-GlcNAcase, and GlcNAc phosphotransferase. The invention relates that said GlcNAc phosphotransferase comprises an .alpha. and .beta. subunit, which reduces substrate specificity, and allows the GlcNAc phosphotransferase to catalyze the transfer of N-acetylglucosamine-1-phosphate from UDP-GlcNAc to the GBA enzyme. The invention discussed that in this method GBA will be phosphorylated which will allow binding to mannose 6 receptors on the surface of lung and bone cells. In so binding to the receptor on these tissues, the problems of the current GBA replacement therapy can be addressed.

L20 ANSWER 2 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:511964 HCAPLUS
DOCUMENT NUMBER: 139:84071
TITLE: Method of producing glycoproteins having reduced complex carbohydrates in mammalian cells
INVENTOR(S): Canfield, William M.
PATENT ASSIGNEE(S): Novazyme Pharmaceuticals, Inc., USA
SOURCE: U.S. Pat. Appl. Publ., 46 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003124653	A1	20030703	US 2001-23890	20011221
WO 2003057828	A2	20030717	WO 2002-US38977	20021220
WO 2003057828	A3	20031120		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-23890 A 20011221
AB The present invention provides a method of producing glycoproteins having reduced complex carbohydrates in a mammalian cell, glycoproteins produced by the method and cells that produce the glycoproteins.

L20 ANSWER 3 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:511963 HCAPLUS
DOCUMENT NUMBER: 139:84070
TITLE: Methods of producing high mannose glycoproteins in complex carbohydrate deficient cells
INVENTOR(S): Canfield, William M.
PATENT ASSIGNEE(S): Novazyme Pharmaceuticals, Inc., USA
SOURCE: U.S. Pat. Appl. Publ., 46 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003124652	A1	20030703	US 2001-23889	20011221
WO 2003057710	A2	20030717	WO 2002-US37618	20021219
WO 2003057710	A3	20041111		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-23889 A 20011221

AB The present invention provides a method for producing high mannose glycoproteins in complex carbohydrate deficient cells and the glycoproteins obtained therein.

L20 ANSWER 4 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:646182 HCAPLUS
DOCUMENT NUMBER: 138:182906
TITLE: Human mannose 6-phosphate-uncovering enzyme is synthesized as a proenzyme that is activated by the endoprotease furin
AUTHOR(S): Do, Hung; Lee, Wang-Sik; Ghosh, Pradipta; Hollowell, Tracy; Canfield, William; Kornfeld, Stuart
CORPORATE SOURCE: Novazyme Pharmaceuticals, Incorporated, Oklahoma City, OK, 73104, USA
SOURCE: Journal of Biological Chemistry (2002), 277(33), 29737-29744
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **N-Acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**, also known as "uncovering" enzyme (UCE), is localized in the trans-Golgi network, where it removes a covering N-acetylglucosamine from the mannose 6-phosphate recognition market on lysosomal acid hydrolases. Here we show that UCE is synthesized as an inactive proenzyme that is activated by the endoprotease furin, which cleaves an RARLPR .dwnarw. D sequence to release a 24-amino acid propiece. As furin is localized in the trans-Golgi network, newly synthesized UCE is inactive until it reaches this terminal Golgi compartment. LoVo cells (derived from a human colon adenocarcinoma) lack furin activity and have extremely low UCE activity. Addn. of furin to LoVo cell exts. restores UCE activity to normal levels, demonstrating that the UCE proenzyme is stable in this cell type. LoVo cells secrete acid hydrolases with phosphomannose diesters as a consequence of the deficient UCE activity. This demonstrates for the first time that UCE is the only enzyme in these cells capable of efficiently uncovering phosphomannose diesters. UCE also hydrolyzes UDP-GlcNAc, a sugar donor for Golgi N-acetylglucosaminyl-transferases. The fact that UCE is not activated until it reaches the trans-Golgi network may ensure that the pool of UDP-GlcNAc in the Golgi stack is not depleted, thereby maintaining proper oligosaccharide assembly.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 5 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:102761 HCAPLUS
DOCUMENT NUMBER: 136:243730
TITLE: Multiple signals regulate trafficking of the mannose 6-phosphate-uncovering enzyme
AUTHOR(S): Lee, Wang-Sik; Rohrer, Jack; Kornfeld, Rosalind; Kornfeld, Stuart
CORPORATE SOURCE: Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (2002), 277(5), 3544-3551
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The "uncovering enzyme," which catalyzes the second step in the formation of the mannose 6-phosphate recognition marker on lysosomal enzyme oligosaccharides, resides primarily in the trans-Golgi network and cycles between this compartment and the plasma membrane. An anal. of green fluorescent protein-uncovering enzyme chimeras revealed that the transmembrane segment and the first 11 residues of the 41-residue-cytoplasmic tail are sufficient for retention in the trans-Golgi network. The next eight residues (486YAYHPLQE493) facilitate exit from this compartment. Kinetic studies demonstrated that the 488YHPL491 sequence also mediates rapid internalization at the plasma membrane. This motif binds adaptor protein-2 in glutathione S-transferase-uncovering enzyme-cytoplasmic tail pull-down assays, indicating that the uncovering enzyme is endocytosed via clathrin-coated vesicles. Consistent with this finding, endogenous uncovering enzyme was detected in purified clathrin-coated vesicles. The enzyme with a Y486A mutation is internalized normally but accumulates on the cell surface because of increased recycling to the plasma membrane. This residue is required for efficient return of the enzyme from endosomes to the trans-Golgi network. These findings indicate that the YAYHPLQE motif is recognized at several sorting sites, including the trans-Golgi network, the plasma membrane, and the endosome.
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 6 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:506933 HCAPLUS
DOCUMENT NUMBER: 137:290704
TITLE: GlcNAc-1-phosphodiester .alpha.-N-acetylglucosaminidase
AUTHOR(S): Kornfeld, Rosalind
CORPORATE SOURCE: Division of Hematology, Washington University School of Medicine, St. Louis, MO, 63110, USA
SOURCE: Handbook of Glycosyltransferases and Related Genes (2002), 617-622. Editor(s): Taniguchi, Naoyuki; Honke, Koichi; Fukuda, Minoru. Springer-Verlag Tokyo: Tokyo, Japan.
CODEN: 69CUXS; ISBN: 4-431-70311-X
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review on the characteristics of **N-acetylglucosamine -1-phosphodiester .alpha.-N-acetylglucosaminidase**, a type I membrane-spanning glycoprotein enzyme of the Golgi app. It includes a discussion on the enzyme's name and history, enzyme activity assay and substrate specificity, prepn., and biol. aspects.
REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 7 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:208390 HCAPLUS
DOCUMENT NUMBER: 134:248843
TITLE: Use of GlcNAc-phosphotransferase and phosphodiester .alpha.-GlcNAcase in production of highly phosphorylated lysosomal hydrolases useful in treatment of lysosomal storage diseases
INVENTOR(S): Canfield, William M.
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001019955	A2	20010322	WO 2000-US21970	20000914
WO 2001019955	A3	20011004		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6534300	B1	20030318	US 2000-635872	20000810
US 6537785	B1	20030325	US 2000-636077	20000810
US 6642038	B1	20031104	US 2000-636060	20000810
US 6770468	B1	20040803	US 2000-636596	20000810
CA 2383217	AA	20010322	CA 2000-2383217	20000914
AU 2000073303	A5	20010417	AU 2000-73303	20000914
BR 2000014514	A	20020723	BR 2000-14514	20000914
EP 1224266	A2	20020724	EP 2000-961335	20000914
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
JP 2003509043	T2	20030311	JP 2001-523727	20000914
US 2002025550	A1	20020228	US 2001-895072	20010702
US 2002150981	A1	20021017	US 2001-986552	20011109
US 6670165	B2	20031230		
US 2003148460	A1	20030807	US 2002-306686	20021129
US 6828135	B2	20041207		

PRIORITY APPLN. INFO.:	US 1999-153831P	P	19990914
	US 2000-635872	A1	20000810
	US 2000-636596	A3	20000810
	WO 2000-US21970	W	20000914

AB The lysosomal targeting pathway enzymes GlcNAc-phosphotransferase and phosphodiester .alpha.-GlcNAcase and uses in prodn. of highly phosphorylated lysosomal hydrolases that can be used to treat lysosomal storage diseases, are disclosed. Generally, the nucleic acid mols. coding for the enzymes are incorporated into expression vectors that are used to transfect host cells that express the enzymes. The expressed enzymes are recovered using monoclonal antibodies capable of selectively binding to bovine GlcNAc-phosphotransferase and to bovine phosphodiester .alpha.-GlcNAcase. Lysosomal hydrolases having high mannose structures are treated with GlcNAc-phosphotransferase and phosphodiester .alpha.-GlcNAcase resulting in the prodn. of asparagine-linked oligosaccharides that are highly modified with mannose 6-phosphate ("M6P"). The treated hydrolase binds to M6P receptors on the cell membrane and is transported into the cell and delivered to the lysosome where it can perform its normal or a desired function. The highly phosphorylated lysosomal hydrolases are readily taken into the cell and into the lysosome during enzyme replacement therapy procedures.

L20 ANSWER 8 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:800029 HCAPLUS
DOCUMENT NUMBER: 136:50263
TITLE: Lysosomal hydrolase mannose 6-phosphate uncovering enzyme resides in the trans-Golgi network
AUTHOR(S): Rohrer, Jack; Kornfeld, Rosalind
CORPORATE SOURCE: Friedrich Miescher Institut, Basel, 4059, Switz.
SOURCE: Molecular Biology of the Cell (2001), 12(6), 1623-1631
CODEN: MBCEEV; ISSN: 1059-1524
PUBLISHER: American Society for Cell Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A crucial step in lysosomal biogenesis is catalyzed by "uncovering" enzyme (UCE), which removes a covering N-acetylglucosamine from the mannose 6-phosphate (Man-6-P) recognition marker on lysosomal hydrolases. This study shows that UCE resides in the trans-Golgi network (TGN) and cycles between the TGN and plasma membrane. The cytosolic domain of UCE contains two potential endocytosis motifs: 488YHPL and C-terminal 511NPFKD. YHPL is shown to be the more potent of the two in retrieval of UCE from the plasma membrane. A green-fluorescent protein-UCE transmembrane-cytosolic domain fusion protein colocalizes with TGN 46, as does endogenous UCE in HeLa cells, showing that the transmembrane and cytosolic domains det. intracellular location. These data imply that the Man-6-P recognition marker is formed in the TGN, the compartment where Man-6-P receptors bind cargo and are packaged into clathrin-coated vesicles.
REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 9 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:738356 HCAPLUS
DOCUMENT NUMBER: 132:60839
TITLE: Molecular cloning and functional expression of two splice forms of human **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**
AUTHOR(S): Kornfeld, Rosalind; Bao, Ming; Brewer, Kevin; Noll, Carolyn; Canfield, William
CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (1999), 274(46), 32778-32785
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have isolated and sequenced human cDNA and mouse genomic DNA clones encoding **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (phosphodiester .alpha.-GlcNAcase) which catalyzes the second step in the synthesis of the mannose 6-phosphate recognition signal on lysosomal enzymes. The gene is organized into 10 exons. The protein sequence encoded by the clones shows 80% identity between human and mouse phosphodiester .alpha.-GlcNAcase and no homol. to other known proteins. It predicts a type I membrane-spanning glycoprotein of 514 amino acids contg. a 24-amino acid signal sequence, a luminal domain of 422 residues with six potential N-linked glycosylation sites, a single 27-residue transmembrane region, and a 41-residue cytoplasmic tail that contains both a tyrosine-based and an NPF internalization motif. Human brain expressed sequence tags lack a 102-base pair region present in human liver cDNA that corresponds to exon 8 in the genomic DNA and probably arises via alternative splicing. COS cells transfected with the human cDNA expressed 50-100-fold increases in phosphodiester

.alpha.-GlcNAcase activity proving that the cDNA encodes the subunits of the tetrameric enzyme. Transfection with cDNA lacking the 102-base pair region also gave active enzyme. The complete genomic sequence of human phosphodiester .alpha.-GlcNAcase was recently deposited in the data base. It showed that our cDNA clone was missing only the 5'-untranslated region and initiator methionine and revealed that the human genomic DNA has the same exon organization as the mouse gene.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 10 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1998:592720 HCAPLUS

DOCUMENT NUMBER: 129:299526

TITLE: Purification and multimeric structure of bovine **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**

AUTHOR(S): Kornfeld, Rosalind; Bao, Ming; Brewer, Kevin; Noll, Carolyn; Canfield, William M.

CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, MO, 63110, USA

SOURCE: Journal of Biological Chemistry (1998), 273(36), 23203-23210
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **N-Acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (EC 3.1.4.45; phosphodiester .alpha.-GlcNAcase) catalyzes the second step in the synthesis of the mannose 6-phosphate determinant required for efficient intracellular targeting of newly synthesized lysosomal hydrolases to the lysosome. A partially purified prepn. of phosphodiester .alpha.-GlcNAcase from bovine pancreas was used to generate a panel of murine monoclonal antibodies. The anti-phosphodiester .alpha.-GlcNAcase monoclonal antibody UC1 was coupled to a solid support and used to immunopurify the bovine liver enzyme 670,000-fold in 2 steps to apparent homogeneity with an overall yield of 14%. The purified phosphodiester .alpha.-GlcNAcase has a specific activity of 498 .mu.mol of [3H]GlcNAc-.alpha.-phosphomannose-.alpha.-Me cleaved per h per mg of protein using 0.5 mM [3H]GlcNAc-.alpha.-phosphomannose-.alpha.-Me as substrate. The subunit structure of the enzyme was detd. using a combination of anal. gel filtration chromatog., SDS-PAGE, and N-terminal sequencing. The data indicate that bovine phosphodiester .alpha.-GlcNAcase is a 272,000-Da complex of 4 identical 68,000-Da glycoprotein subunits arranged as 2 disulfide-linked homodimers. A sol. form of the enzyme, isolated from fetal bovine serum, showed the same subunit structure. Both forms of the enzyme reacted with a rabbit antibody raised to the N-terminal peptide of the liver enzyme, suggesting that phosphodiester .alpha.-GlcNAcase is a type I membrane-spanning glycoprotein with its N-terminus in the lumen of the Golgi app.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 11 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1996:666410 HCAPLUS

DOCUMENT NUMBER: 125:321280

TITLE: Purification and characterization of human lymphoblast **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**

AUTHOR(S): Page, Theodore; Zhao, Ke-Wei; Tao, Ling; Miller, Arnold L.

CORPORATE SOURCE: Department of Neurosciences 0624, University of

California, San Diego, La Jolla, CA, 92093-0624, USA
SOURCE: Glycobiology (1996), 6(6), 619-626
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (EC 3.1.4.45; uncovering enzyme) (I) catalyzes the removal of N-acetylglucosamine from the N-acetylglucosamine-.alpha.-phospho-mannose portion of selected lysosomal enzyme oligosaccharide chains, thereby forming the mannose 6-phosphate signal which is responsible for the targeting of these lysosomal enzymes for transport into lysosomes. I was purified .apprx.7000-fold to electrophoretic homogeneity from Epstein-Barr virus-transformed human lymphoblast cells. The purifn. sequence involved solubilizing this membrane-bound enzyme with Tergitol NP-10, affinity chromatog. on lentil lectin-Sepharose 4B, ion-exchange chromatog. on DEAE-Sephacel, chromatog. on Zn(II)-IDA-Sepharose 6B, and preparative SDS-PAGE electrophoresis. Purified I migrated as a single band of 114 kDa which was coincident with enzyme activity on anal. SDS-PAGE electrophoresis. Characterization studies of purified I demonstrated that catalytic activity was maximal at pH 6.95 and that the enzyme retained full activity following incubation for 10 min at 60.degree.. No requirement was found for a divalent cation, but Zn2+, Hg2+, and Cu2+ were found to reduce I activity by 30-40%. The highest catalytic efficiency was obsd. with N-acetylglucosamine-phospho-methylmannoside as a substrate, whereas UDP-N-acetylglucosamine, N-acetylglucosamine-phosphomannose-uteroferrin, and N-acetylglucosamine phosphate were also cleaved by I with decreasing efficiency. 6-Acetamido-6-deoxycastanospermine was a potent inhibitor of human I with a Ki of 0.35 .mu.M, whereas N-acetylglucosamine phosphate (Ki = 1.58 mM) and N-acetylglucosamine (Ki = 5.1 mM) inhibited I to a lesser degree.

L20 ANSWER 12 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:620244 HCAPLUS
DOCUMENT NUMBER: 123:50595
TITLE: Purification and characterization of human serum **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**
AUTHOR(S): Lee, Jin Kyu; Pierce, Michael
CORPORATE SOURCE: Complex Carbohydrate Res. Center, Univ. Georgia, Athens, GA, 30602, USA
SOURCE: Archives of Biochemistry and Biophysics (1995), 319(2), 413-25
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Many lysosomal enzymes are recognized and selected by a unique marker in the form of mannose 6-phosphate groups which are present exclusively on their N-linked oligosaccharides. Two enzymes act sequentially to catalyze the addn. of mannose 6-phosphate groups to the proteins: N-acetylglucosamine phosphotransferase and **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (I). Here, the purifn. and partial characterization of I from human blood serum is reported. I was purified >600,000-fold by utilizing (NH4)2SO4 pptn., fractionation on wheat germ agglutinin-Sepharose, Fe3+-chelating Sepharose, and Cu2+-chelating Sepharose, and renaturation from gel slices after SDS-PAGE. I obsd. after renaturation and subsequent SDS-PAGE and Ag staining had an apparent mol. wt. of 118 kDa, which was slightly smaller than bovine liver I. Serum I activity did not require Triton X-100 and was not stimulated by its addn. These results suggest that I found in serum represents a form secreted after proteolysis in the Golgi app. of

the membrane-bound enzyme. Serum I hydrolyzed UDP-GlcNAc to UDP and GlcNAc and hydrolyzed GlcNAc-P-Man.alpha.Me into .alpha.MeMan-P and GlcNAc. I had no hydrolytic activity toward UDP-GalNAc, UDP-Glc, [6-3H]GlcNAc.beta.1-3Gal.beta.1-4Glc, p-nitrophenyl-.alpha.-N-acetylglucosaminide, p-nitrophenyl-.beta.-N-acetylglucosamide, p-nitrophenyl-.alpha.-N-galactopyranoside, or p-nitrophenyl-.beta.-N-galactopyranoside. I was strongly inhibited by UDP-GlcNAc and GlcNAc-1-phosphate, had a pH optimum of 6.0-7.0, and was inhibited by FeCl3, FeSO4, and CuSO4. The Km values for UDP-GlcNAc and GlcNAc-P-Man.alpha.Me were 0.94 and 0.45 mM, resp. Over 77% of I activity remained after incubation for 10 min at 70.degree., demonstrating an unusual thermostability of the serum enzyme.

L20 ANSWER 13 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:376010 HCAPLUS
DOCUMENT NUMBER: 123:4103
TITLE: Lysosomal enzyme targeting. Purification and characterization of the two enzymes that catalyze the formation of mannose-6-phosphate from human lymphoblasts
AUTHOR(S): Zhao, Ke-Wei
CORPORATE SOURCE: Univ. Calif., San Diego, CA, USA
SOURCE: (1994) 160 pp. Avail.: Univ. Microfilms Int., Order No. DA9422574
From: Diss. Abstr. Int. B, 1994, 55(3), 743
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L20 ANSWER 14 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1994:128269 HCAPLUS
DOCUMENT NUMBER: 120:128269
TITLE: Characterization and immunolocalization of bovine **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**
AUTHOR(S): Mullis, Karen Gheesling; Kornfeld, Rosalind H.
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (1994), 269(3), 1727-33
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (phosphodiester .alpha.-GlcNAcase) has been purified 3,000-fold from bovine liver and its kinetic properties detd. as described in the previous report (Mullis, K. G., Huynh, M., and Kornfeld, R. (1993) J. Biol. Chem. 269, 1718-1726). This report describes the hydrodynamic and lectin binding properties of phosphodiester .alpha.-GlcNAcase as well as its intracellular localization. The mol. wt. of phosphodiester .alpha.-GlcNAcase is 204,950, as detd. from d. gradient centrifugation in D2O and H2O glycerol gradients and gel filtration. Enzymically active enzyme migrates on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a mol. wt. of 129,000, consistent with native phosphodiester .alpha.-GlcNAcase being a dimer. The lectin binding properties of phosphodiester .alpha.-GlcNAcase indicate that it contains sialylated species of both complex type N-linked oligosaccharides and O-linked oligosaccharides. In immunofluorescence studies phosphodiester .alpha.-GlcNAcase shows a perinuclear, Golgi localization in Vero cells as does the mid-Golgi marker .alpha.-mannosidase II. After exposure of the Vero cells to brefeldin A, phosphodiester .alpha.-GlcNAcase assumes an endoplasmic reticulum staining pattern. In contrast, in cells costained with the trans-Golgi marker wheat germ agglutinin, the wheat germ agglutinin marker assumed an endosomal network appearance after exposure

to brefeldin A. These findings indicate that phosphodiester .alpha.-GlcNAcase is normally located within the Golgi stack, sep. from the trans-Golgi and trans-Golgi network stained by wheat germ agglutinin.

L20 ANSWER 15 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1994:264244 HCAPLUS
DOCUMENT NUMBER: 120:264244
TITLE: Purification and kinetic parameters of bovine liver **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**
AUTHOR(S): Mullis, Karen Gheesling; Huynh, Michiko; Kornfeld, Rosalind H.
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (1994), 269(3), 1718-26
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The enzyme N-acetylglucosamine phosphodiester .alpha.-N-acetylglucosaminidase (phosphodiester .alpha.-GlcNAcase) catalyzes the second step in the formation of the mannose 6-phosphate targeting signal on lysosomal enzyme oligosaccharides by removing GlcNAc residues from GlcNAc-.alpha.-P-mannose moieties, which are formed in the first step by UDP-N-acetylglucosamine:glycoprotein N-acetylglucosamine-1-phosphotransferase (GlcNAc-phosphotransferase). Phosphodiester .alpha.-GlcNAcase, a membrane-bound enzyme, has been purified about 3,000-fold from bovine liver to apparent homogeneity using detergent solubilization, fractionation on DEAE-cellulose, affinity chromatog. on lectin-Sepharose columns, gel filtration, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The enzyme migrated as 129- and 121-kDa species on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Since both bands had the same amino-terminal sequence, the smaller species is presumed to be derived from the larger by proteolysis. Kinetic anal. of bovine phosphodiester .alpha.-GlcNAcase with enzymically synthesized artificial and biol. substrates indicates that phosphodiester .alpha.-GlcNAcase requires GlcNAc-.alpha.-P R for substrate and that when R contains the Man.alpha.1,2Man linkage the substrate binding is most effective. Unlike GlcNAc-phosphotransferase, bovine phosphodiester .alpha.-GlcNAcase does not require a protein recognition determinant on lysosomal enzyme substrates.

L20 ANSWER 16 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:151992 HCAPLUS
DOCUMENT NUMBER: 126:182923
TITLE: **N-Acetylglucosamine-1-phosphodiester-.alpha.-N-acetylglucosaminidase**
AUTHOR(S): Mullis, Karen G.
CORPORATE SOURCE: Division of Hematology/Oncology, Washington Univ. Sch. of Medicine, St. Louis, MO, 63110-1093, USA
SOURCE: Guidebook to the Secretory Pathway (1994), 278-279. Editor(s): Rothblatt, Jonathan; Novick, Peter; Stevens, Tom H. Oxford University Press: Oxford, UK.
CODEN: 64AJAT
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review with 6 refs. **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (uncovering enzyme; .alpha.-acetylglucosaminyloligosaccharide phosphodiesterase; EC 3.1.4.45) is a membrane-bound Golgi enzyme that removes the N-acetylglucosamine residue from N-acetylglucosamine-phosphate-mannose (GlcNAc-P-Man) groups on oligosaccharides of newly synthesized lysosomal enzymes. The resulting mannose 6-phosphate (Man 6-P) serves as the recognition marker for binding

Man 6-P receptors and transport to the lysosomes.

L20 ANSWER 17 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:151991 HCAPLUS
DOCUMENT NUMBER: 126:182922
TITLE: UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine 1-phosphotransferase
AUTHOR(S): Ketcham, Catherine
CORPORATE SOURCE: Division of Hematology/Oncology, Washington Univ. Sch. of Medicine, St. Louis, MO, 63110-1093, USA
SOURCE: Guidebook to the Secretory Pathway (1994), 277-278. Editor(s): Rothblatt, Jonathan; Novick, Peter; Stevens, Tom H. Oxford University Press: Oxford, UK. CODEN: 64AJAT
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review with 18 refs. UDP-N-acetylglucosamine-lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (EC 2.7.8.17) transfers N-acetylglucosamine 1-phosphate from UDP-N-acetylglucosamine to certain mannose residues of lysosomal enzymes. The covering GlcNAc residue is then removed by **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**, resulting in generation of mannose 6-phosphate, the lysosomal enzyme recognition marker. Phosphorylated lysosomal enzymes then bind to mannose 6-phosphate receptors for subsequent targeting to lysosomes.

L20 ANSWER 18 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:423458 HCAPLUS
DOCUMENT NUMBER: 119:23458
TITLE: Characterization of UDP-N-acetylglucosamine:glycoprotein N-acetylglucosamine-1-phosphotransferase from Acanthamoeba castellanii
AUTHOR(S): Ketcham, Catherine M.; Kornfeld, Stuart
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (1992), 267(16), 11654-9 CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The kinetic properties of UDP-N-acetylglucosamine-glycoprotein N-acetylglucosamine-1-phosphotransferase (I) partially purified from A. castellanii were studied. I phosphorylated the lysosomal enzymes, uteroferrin (acid phosphatase) and cathepsin D, 3-90-fold better than nonlysosomal glycoproteins and 16-83-fold better than a Man9GlcNAc oligosaccharide. Deglycosylated uteroferrin was a potent competitive inhibitor of the phosphorylation of intact uteroferrin (Ki = 48 .mu.M) but did not inhibit the phosphorylation of RNase B or the simple sugar, .alpha.-methylmannoside. Deglycosylated RNase A did not inhibit the phosphorylation of RNase B or uteroferrin. The results indicated that purified I recognizes a protein domain present on lysosomal enzymes but absent in most nonlysosomal glycoproteins. I also exhibited a marked preference for oligosaccharides contg. mannose .alpha.1,2-mannose sequences, but this could not account for the high-affinity binding to lysosomal enzymes. A. castellanii exts. did not contain detectable levels of **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**, the 2nd enzyme in the biosynthetic pathway for the mannose 6-phosphate recognition marker. Thus, A. castellanii does not utilize the phosphomannosyl sorting pathway despite expression of very high levels of I.

L20 ANSWER 19 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:163736 HCAPLUS
DOCUMENT NUMBER: 118:163736

TITLE: The synthesis of substrates and two assays for the detection of **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (uncovering enzyme)
AUTHOR(S): Mullis, Karen Gheesling; Ketcham, Catherine M.
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SOURCE: Analytical Biochemistry (1992), 205(2), 200-7
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A method for the synthesis and purifn. of large quantities of 4 radiolabeled substrates for quantitation of **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (uncovering enzyme) (I) is described. Four substrates, [3H]GlcNAc-.alpha.-P-Man.alpha.Me, [3H]GlcNAc-.alpha.-P-uteroferrin, [3H]GlcNAc.alpha.-P-Man.alpha.1-2Man-O-Me, and [3H]GlcNAc.alpha.-P-Man9GlcNAc, were enzymically synthesized using acetylglucosaminephosphotransferase from Acanthamoeba castellanii and UDP-N-acetyl-[3H]glucosamine and, as acceptor, methyl-.alpha.-D-mannopyranoside (Man.alpha.Me), uteroferrin, Man.alpha.1-2Man-O-Me, or Man9GlcNAc. The isolation of the [3H]GlcNAc-P-modified product of each reaction is detailed. Two assays for the detection of I activity using [3H]GlcNAc-.alpha.-P-uteroferrin and [3H]GlcNAc-.alpha.-P-Man.alpha.Me are outlined. The ability to easily synthesize 4 relevant substrates for I offers flexibility in assaying I.

L20 ANSWER 20 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1991:181592 HCAPLUS
DOCUMENT NUMBER: 114:181592
TITLE: The isolated ER-Golgi intermediate compartment exhibits properties that are different from ER and cis-Golgi
AUTHOR(S): Schweizer, Anja; Matter, Karl; Ketcham, Catherine M.; Hauri, Hans Peter
CORPORATE SOURCE: Biocent., Univ. Basel, Basel, CH-4056, Switz.
SOURCE: Journal of Cell Biology (1991), 113(1), 45-54
CODEN: JCLBA3; ISSN: 0021-9525
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A procedure has been established in Vero cells for the isolation of an intermediate compartment involved in protein transport from the endoplasmic reticulum (ER) to the Golgi app. The two-step subcellular fractionation procedure consists of Percoll followed by Metrizamide gradient centrifugation. Using the previously characterized p53 as a marker protein, the av. enrichment factor of the intermediate compartment was 41. The purified fraction displayed a unique polypeptide pattern. It was largely sepd. from the rough ER proteins ribophorin I, ribophorin II, BIP, and protein disulfide isomerase, as well as from the putative cis-Golgi marker **N-acetylglucosamine-1-phosphodiester-.alpha.-N-acetylglucosaminidase**, the second of the two enzymes generating the lysosomal targeting signal mannose-6-phosphate. The first enzyme, N-acetylglucosaminylphosphotransferase, for which previous biochem. evidence had suggested both a pre- and a cis-Golgi localization in other cell types, cofractionated with the cis-Golgi rather than the intermediate compartment in Vero cells. The results suggest that the intermediate compartment defined by p53 has unique properties and does not exhibit typical features of rough ER and cis-Golgi.

L20 ANSWER 21 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1990:547654 HCAPLUS
DOCUMENT NUMBER: 113:147654
TITLE: Suppression of the 'uncovering' of mannose-6-phosphate residues in lysosomal enzymes in the presence of

ammonium chloride
AUTHOR(S): Isidoro, Ciro; Radons, Juergen; Baccino, Francesco M.; Hasilik, Andrej
CORPORATE SOURCE: Inst. Physiol. Chem. Pathobiochem., Westfael. Wilhelms-Univ., Muenster, D-4400, Germany
SOURCE: European Journal of Biochemistry (1990), 191(3), 591-7
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The uncovering ratio of phosphate groups in lysosomal enzymes is defined as the percentage of phosphomonoester groups in the oligosaccharide side chains based on the sum of phosphomonoester and phosphodiester groups. Using a new procedure for the specific and complete hydrolysis of uncovered phosphomonoester groups in denatured immunoppts. of human cathepsin D. It is shown that the uncovering ratio varies between different forms of the enzyme and may be used as an indicator of the maturation of its carbohydrate side chains. The uncovering ratio in the total (cellular and secreted) cathepsin D from U937 promonocytes is >95%. It is only slightly decreased in cells incubated in the presence of 1.alpha.,25-dihydroxycholecalciferol, in which the rate of synthesis of cathepsin D is several-times-higher than in the control cells. In U937 cells and also in fibroblasts, the uncovering is nearly complete in mature forms of the intracellular cathepsin D but less extensive in the intracellular and secreted precursor. In both cell types, incubation with 10 mM NH4Cl results in a decrease in the uncovering ratio of total cathepsin D. However, the activity of the uncovering enzyme, **N-acetylglucosamine-1-phosphodiester alpha.-N-acetylglucosaminidase**, as detd. with UDP-N-acetylglucosamine is not affected with up to 60 mM NH4Cl. These results suggest that NH4Cl, in addn. to its known effects on the acidic-pH-dependent functions of lysosomal compartments and of mannose-6-phosphate receptors, impairs the processing or transport of lysosomal enzyme precursors at, or proximally to, the site of the uncovering of their mannose-6-phosphate residues.

L20 ANSWER 22 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1983:158284 HCAPLUS
DOCUMENT NUMBER: 98:158284
TITLE: Evidence for extensive subcellular organization of asparagine-linked oligosaccharide processing and lysosomal enzyme phosphorylation
AUTHOR(S): Goldberg, Daniel E.; Kornfeld, Stuart
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (1983), 258(5), 3159-65
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Membranes prepd. from mouse lymphoma BW5147.3 cells and P388D1 macrophages were fractionated on a continuous sucrose gradient and assayed for enzymes involved in the processing of asparagine-linked oligosaccharides. The order in which these enzymes distributed from dense to light membranes correlated with the established sequence of events in glycoprotein biosynthesis. A no. of enzymes which have been previously localized to the Golgi sepd. into 4 regions on the gradient. UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase, the enzyme which catalyzes the selective phosphorylation of the high mannose oligosaccharides of lysosomal enzymes, was present in the densest membranes. **N-Acetylglucosamine 1-phosphodiester alpha.-N-acetylglucosaminidase** was in the next region. Several enzymes involved in the late stages of asparagine-linked oligosaccharide processing were localized to the 3rd region. UDP-galactose:N-acetylglucosamine galactosyltransferase was present in the lightest membranes (region IV). Pulse-chase expts. utilizing

[2-3H]mannose demonstrated that the distribution of in vivo labeled asparagine-linked oligosaccharide intermediates correlates with the distribution of these processing enzymes. Anal. of the phosphorylated oligosaccharides of lysosomal enzymes which were bound to the phosphomannosyl receptor indicated that these enzymes had already passed through the region of the Golgi which contains galactosyltransferase and sialyltransferase. These findings are consistent with there being a high degree of organization within the Golgi complex. The phys. sepn. of processing enzymes could serve as one mechanism for the control of asparagine-linked oligosaccharide biosynthesis.

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=> s l20 and purification
L21          22 S L20
         302298 PURIFICATION
L22           5 L21 AND PURIFICATION

=> d l22 1-5 ibib ab

L22  ANSWER 1 OF 5  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         1998:592720  HCAPLUS
DOCUMENT NUMBER:          129:299526
TITLE:                    Purification and multimeric structure of
                          bovine N-acetylglucosamine-
                          1-phosphodiester .alpha.-
                          N-acetylglucosaminidase
AUTHOR(S):                Kornfeld, Rosalind; Bao, Ming; Brewer, Kevin; Noll,
                          Carolyn; Canfield, William M.
CORPORATE SOURCE:         Department of Medicine, Washington University School
                          of Medicine, St. Louis, MO, 63110, USA
SOURCE:                   Journal of Biological Chemistry (1998), 273(36),
                          23203-23210
                          CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER:                American Society for Biochemistry and Molecular
                          Biology
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   N-Acetylglucosamine-1-phosphodiester
     .alpha.-N-acetylglucosaminidase (EC
     3.1.4.45; phosphodiester .alpha.-GlcNAcase) catalyzes the second step in
     the synthesis of the mannose 6-phosphate determinant required for
     efficient intracellular targeting of newly synthesized lysosomal
     hydrolases to the lysosome. A partially purified prepn. of phosphodiester
     .alpha.-GlcNAcase from bovine pancreas was used to generate a panel of
     murine monoclonal antibodies. The anti-phosphodiester .alpha.-GlcNAcase
     monoclonal antibody UC1 was coupled to a solid support and used to
     immunopurify the bovine liver enzyme 670,000-fold in 2 steps to apparent
     homogeneity with an overall yield of 14%. The purified phosphodiester
     .alpha.-GlcNAcase has a specific activity of 498 .mu.mol of
     [3H]GlcNAc-.alpha.-phosphomannose-.alpha.-Me cleaved per h per mg of
     protein using 0.5 mM [3H]GlcNAc-.alpha.-phosphomannose-.alpha.-Me as
     substrate. The subunit structure of the enzyme was detd. using a
     combination of anal. gel filtration chromatog., SDS-PAGE, and N-terminal
     sequencing. The data indicate that bovine phosphodiester
     .alpha.-GlcNAcase is a 272,000-Da complex of 4 identical 68,000-Da
     glycoprotein subunits arranged as 2 disulfide-linked homodimers. A sol.
     form of the enzyme, isolated from fetal bovine serum, showed the same
     subunit structure. Both forms of the enzyme reacted with a rabbit
     antibody raised to the N-terminal peptide of the liver enzyme, suggesting
     that phosphodiester .alpha.-GlcNAcase is a type I membrane-spanning
     glycoprotein with its N-terminus in the lumen of the Golgi app.
REFERENCE COUNT:          15    THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22  ANSWER 2 OF 5  HCAPLUS  COPYRIGHT 2004 ACS on STN
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ACCESSION NUMBER: 1996:666410 HCAPLUS
DOCUMENT NUMBER: 125:321280
TITLE: **Purification** and characterization of human lymphoblast **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**
AUTHOR(S): Page, Theodore; Zhao, Ke-Wei; Tao, Ling; Miller, Arnold L.
CORPORATE SOURCE: Department of Neurosciences 0624, University of California, San Diego, La Jolla, CA, 92093-0624, USA
SOURCE: Glycobiology (1996), 6(6), 619-626
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (EC 3.1.4.45; uncovering enzyme) (I) catalyzes the removal of N-acetylglucosamine from the N-acetylglucosamine-.alpha.-phospho-mannose portion of selected lysosomal enzyme oligosaccharide chains, thereby forming the mannose 6-phosphate signal which is responsible for the targeting of these lysosomal enzymes for transport into lysosomes. I was purified .apprx.7000-fold to electrophoretic homogeneity from Epstein-Barr virus-transformed human lymphoblast cells. The purifn. sequence involved solubilizing this membrane-bound enzyme with Tergitol NP-10, affinity chromatog. on lentil lectin-Sepharose 4B, ion-exchange chromatog. on DEAE-Sephacel, chromatog. on Zn(II)-IDA-Sepharose 6B, and preparative SDS-PAGE electrophoresis. Purified I migrated as a single band of 114 kDa which was coincident with enzyme activity on anal. SDS-PAGE electrophoresis. Characterization studies of purified I demonstrated that catalytic activity was maximal at pH 6.95 and that the enzyme retained full activity following incubation for 10 min at 60.degree.. No requirement was found for a divalent cation, but Zn2+, Hg2+, and Cu2+ were found to reduce I activity by 30-40%. The highest catalytic efficiency was obsd. with N-acetylglucosamine-phospho-methylmannoside as a substrate, whereas UDP-N-acetylglucosamine, N-acetylglucosamine-phosphomannose-uteroferrin, and N-acetylglucosamine phosphate were also cleaved by I with decreasing efficiency. 6-Acetamido-6-deoxycastanospermine was a potent inhibitor of human I with a Ki of 0.35 .mu.M, whereas N-acetylglucosamine phosphate (Ki = 1.58 mM) and N-acetylglucosamine (Ki = 5.1 mM) inhibited I to a lesser degree.

L22 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:620244 HCAPLUS
DOCUMENT NUMBER: 123:50595
TITLE: **Purification** and characterization of human serum **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**
AUTHOR(S): Lee, Jin Kyu; Pierce, Michael
CORPORATE SOURCE: Complex Carbohydrate Res. Center, Univ. Georgia, Athens, GA, 30602, USA
SOURCE: Archives of Biochemistry and Biophysics (1995), 319(2), 413-25
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Many lysosomal enzymes are recognized and selected by a unique marker in the form of mannose 6-phosphate groups which are present exclusively on their N-linked oligosaccharides. Two enzymes act sequentially to catalyze the addn. of mannose 6-phosphate groups to the proteins: N-acetylglucosamine phosphotransferase and **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase** (I). Here, the

purifn. and partial characterization of I from human blood serum is reported. I was purified >600,000-fold by utilizing (NH4)2SO4 pptn., fractionation on wheat germ agglutinin-Sepharose, Fe3+-chelating Sepharose, and Cu2+-chelating Sepharose, and renaturation from gel slices after SDS-PAGE. I obsd. after renaturation and subsequent SDS-PAGE and Ag staining had an apparent mol. wt. of 118 kDa, which was slightly smaller than bovine liver I. Serum I activity did not require Triton X-100 and was not stimulated by its addn. These results suggest that I found in serum represents a form secreted after proteolysis in the Golgi app. of the membrane-bound enzyme. Serum I hydrolyzed UDP-GlcNAc to UDP and GlcNAc and hydrolyzed GlcNAc-P-Man.alpha.Me into .alpha.MeMan-P and GlcNAc. I had no hydrolytic activity toward UDP-GalNAc, UDP-Glc, [6-3H]GlcNAc.beta.1-3Gal.beta.1-4Glc, p-nitrophenyl-.alpha.-N-acetylglucosaminide, p-nitrophenyl-.beta.-N-acetylglucosamide, p-nitrophenyl-.alpha.-N-galactopyranoside, or p-nitrophenyl-.beta.-N-galactopyranoside. I was strongly inhibited by UDP-GlcNAc and GlcNAc-1-phosphate, had a pH optimum of 6.0-7.0, and was inhibited by FeCl3, FeSO4, and CuSO4. The Km values for UDP-GlcNAc and GlcNAc-P-Man.alpha.Me were 0.94 and 0.45 mM, resp. Over 77% of I activity remained after incubation for 10 min at 70.degree., demonstrating an unusual thermostability of the serum enzyme.

L22 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:376010 HCAPLUS
DOCUMENT NUMBER: 123:4103
TITLE: Lysosomal enzyme targeting. **Purification** and characterization of the two enzymes that catalyze the formation of mannose-6-phosphate from human lymphoblasts
AUTHOR(S): Zhao, Ke-Wei
CORPORATE SOURCE: Univ. Calif., San Diego, CA, USA
SOURCE: (1994) 160 pp. Avail.: Univ. Microfilms Int., Order No. DA9422574
From: Diss. Abstr. Int. B, 1994, 55(3), 743
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L22 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1994:264244 HCAPLUS
DOCUMENT NUMBER: 120:264244
TITLE: **Purification** and kinetic parameters of bovine liver **N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase**
AUTHOR(S): Mullis, Karen Gheesling; Huynh, Michiko; Kornfeld, Rosalind H.
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (1994), 269(3), 1718-26
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The enzyme N-acetylglucosamine phosphodiester .alpha.-N-acetylglucosaminidase (phosphodiester .alpha.-GlcNAcase) catalyzes the second step in the formation of the mannose 6-phosphate targeting signal on lysosomal enzyme oligosaccharides by removing GlcNAc residues from GlcNAc-.alpha.-P-mannose moieties, which are formed in the first step by UDP-N-acetylglucosamine:glycoprotein N-acetylglucosamine-1-phosphotransferase (GlcNAc-phosphotransferase). Phosphodiester .alpha.-GlcNAcase, a membrane-bound enzyme, has been purified about 3,000-fold from bovine liver to apparent homogeneity using detergent solubilization, fractionation on DEAE-cellulose, affinity chromatog. on lectin-Sepharose columns, gel filtration, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The enzyme migrated as 129-

and 121-kDa species on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Since both bands had the same amino-terminal sequence, the smaller species is presumed to be derived from the larger by proteolysis. Kinetic anal. of bovine phosphodiester .alpha.-GlcNAcase with enzymically synthesized artificial and biol. substrates indicates that phosphodiester .alpha.-GlcNAcase requires GlcNAc-.alpha.-P R for substrate and that when R contains the Man.alpha.1,2Man linkage the substrate binding is most effective. Unlike GlcNAc-phosphotransferase, bovine phosphodiester .alpha.-GlcNAcase does not require a protein recognition determinant on lysosomal enzyme substrates.

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=>  d his

     (FILE 'HOME' ENTERED AT 10:48:21 ON 10 DEC 2004)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, EMBASE, SCISEARCH' ENTERED AT
     10:48:55 ON 10 DEC 2004
L1           411 S N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L2           184 DUP REM L1 (227 DUPLICATES REMOVED)
L3           146 S L2 AND 1975-1999/PY
L4             4 S HUMAN N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L5             4 DUP REM L4 (0 DUPLICATES REMOVED)
L6             6 S (HUMAN N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE OR HUMAN GLCN
L7             6 DUP REM L6 (0 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 10:54:39 ON 10 DEC 2004
L8           105 S REGISTRY

     FILE 'REGISTRY' ENTERED AT 10:56:18 ON 10 DEC 2004
L9             0 S HUMAN N-ACETYLGLUCOSAMINE-      1-PHOSPHOTRANSFERASE
L10            3 S  N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE
L11            5 S GLCNAC-PHOSPHOTRANSFERASE
L12            0 S  N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER N-ACETYLGLUCOSAMINIDASE
L13            0 S PHOSPHODIESTER-GLCNAASE
L14            0 S PHOSPHODIESTER-GLCNACASE
L15            0 S PHOSPHODIESTER-GLCNACASE

     FILE 'HCAPLUS' ENTERED AT 11:05:26 ON 10 DEC 2004
L16            2 S N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER N-ACETYLGLUCOSAMINIDASE
L17            2 DUP REM L16 (0 DUPLICATES REMOVED)
L18            0 S PHOSPHODIESTER GLCNACASE
L19           22 S N-ACETYLGLUCOSAMINE-1-PHOSPHODIESTER ALPHA- N-ACETYLGLUCOSAMI
L20           22 DUP REM L19 (0 DUPLICATES REMOVED)
L21           22 S L20
L22            5 S L20 AND PURIFICATION

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                   101.65     253.31

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                   -18.90     -19.60

STN INTERNATIONAL LOGOFF AT 11:13:24 ON 10 DEC 2004
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=> s N-ACETYLGLUCOSAMINE-1-PHOSPHOtransferase and 1975-1999/py
   3 FILES SEARCHED...
L1          282 N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE AND 1975-1999/PY

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2           118 DUP REM L1 (164 DUPLICATES REMOVED)

=> s l2 and (purified or purification or isolation)
L3           25 L2 AND (PURIFIED OR PURIFICATION OR ISOLATION)

=> focus l3
PROCESSING COMPLETED FOR L3
L4            25 FOCUS L3 1-

=> d l4 1-25 ibib ab

L4   ANSWER 1 OF 25  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         1994:674939  HCAPLUS
DOCUMENT NUMBER:          121:274939
TITLE:                    Mouse polyclonal antibodies to UDP-N-
                          acetylglucosamine:lysosomal enzyme N-
                          acetylglucosamine-1-
                          phosphotransferase from Acanthamoeba
                          castellanii
AUTHOR(S):                Guillen, E.; Cafferata, E. G. A.; Couso, R. O.
CORPORATE SOURCE:         Inst. de Invest. Bioquim. <<Fundacion Compomar>> Av.
                          Patricias Argent., Buenos Aires, (1405), Argent.
SOURCE:                   Anales de la Asociacion Quimica Argentina (
                          1993), 81(2-3), 133-46
                          CODEN: AAQAAE; ISSN: 0365-0375
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   Mice were immunized with prepns. of lysosomal enzyme
     acetylglucosaminephosphotransferase (EC 2.7.8.17) (I) purified
     >10,000-fold. Specific antibodies for A. castellanii proteins and with
     high affinity for I were obtained. Crude and highly purified I
     prepns. were chromatographed on an exclusion mol. column, in which the
     enzyme presented elution vols. that depended on the prepns. The proteins
     of the eluted fractions were sepd. by electrophoresis and immunoblots
     developed with the mouse antiserum. Anal. of the proteins that reacted
     with the antibodies and whose intensity profiles correlated with I
     activity, showed that 2 proteins of 71 and 82 kDa were probably subunits
     or subunit fragments of I.

L4   ANSWER 2 OF 25     MEDLINE on STN
ACCESSION NUMBER:   95221381     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 7706268
TITLE:              Characterization and partial purification of a
                    novel enzymatic activity. UDP-GlcNAc:Ser-protein N
                    -acetylglucosamine-1-
                    phosphotransferase from the cellular slime mold
                    Dictyostelium discoideum.
AUTHOR:             Merello S; Parodi A J; Couso R
CORPORATE SOURCE:   Instituto de Investigaciones Bioquimicas Fundacion
                    Campomar, Buenos Aires, Argentina.
SOURCE:             Journal of biological chemistry, (1995 Mar 31)
                    270 (13) 7281-7.
                    Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        199505
ENTRY DATE:         Entered STN: 19950518
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Last Updated on STN: 19970203
Entered Medline: 19950510

AB An enzymatic activity that transfers N-acetylglucosamine-1-phosphate residues from UDP-GlcNAc to serine units in proteins (UDP-GlcNAc:Ser-protein **N-acetylglucosamine-1-phosphotransferase**) was detected in membranes of the cellular slime mold Dictyostelium discoideum. The enzyme was partially **purified** by affinity chromatography in concanavalin A-Sepharose and ion exchange chromatography in a Mono Q column. The enzyme showed an absolute requirement for bivalent cations, Mn2+ being more effective than Mg2+. It had a broad optimum pH value (6.5-9.0). The Km for UDP-GlcNAc was 18 microM. In cell free assays it used apomucin and native or 8 M urea-denatured thyroglobulin but neither bovine serum albumin nor native or denatured uteroferrin as exogenous acceptors. Analysis of proteins isolated from cells grown in the presence of [32P]phosphate and from the culture medium showed that the majority of proteins bearing the structure Glc-NAc-1-P-Ser were secreted. In equilibrium density centrifugations of microsomes, the enzyme appeared in membranes having lighter densities than the enzyme that phosphorylates high mannose-type oligosaccharides. This showed that the activity that phosphorylates serine residues in proteins (UDP-GlcNAc:Ser-protein **N-acetylglucosamine-1-phosphotransferase**) is different from that phosphorylating protein-linked high mannose-type oligosaccharides (UDP-GlcNAc:glycoprotein **N-acetylglucosamine-1-phosphotransferase**).

L4 ANSWER 3 OF 25 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1987:209934 HCAPLUS
DOCUMENT NUMBER: 106:209934
TITLE: Lipid-linked intermediates in crustacean chitin synthesis
AUTHOR(S): Horst, M. N.
CORPORATE SOURCE: Sch. Med., Mercer Univ., Macon, GA, 31207, USA
SOURCE: Chitin Nat. Technol., [Proc. Int. Conf. Chitin Chitosan], 3rd (**1986**), Meeting Date 1985, 45-52. Editor(s): Muzzarelli, Riccardo A. A.; Jeuniaux, Charles; Gooday, Graham W. Plenum: New York, N. Y.
CODEN: 55MHAB
DOCUMENT TYPE: Conference
LANGUAGE: English

AB UDP-GlcNAc-dolichol phosphate **N-acetylglucosamine-1-phosphotransferase** (I), partially **purified** from Artemia salina microsomes, catalyzed the formation of the lipid-linked intermediate, dolichol pyrophosphate N-acetylglucosamine. I had Km values for dolichol phosphate and UDP-GlcNAc of 3.3 and 0.17 .mu.M, resp. I was assocd. with phospholipids and was inhibited by tunicamycin (98%), diumycin (30%), and amphomycin.

L4 ANSWER 4 OF 25 MEDLINE on STN
ACCESSION NUMBER: 97094921 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8940155
TITLE: Bovine UDP-N-acetylglucosamine:lysosomal-enzyme **N-acetylglucosamine-1-phosphotransferase**. I. **Purification** and subunit structure.
AUTHOR: Bao M; Booth J L; Elmendorf B J; Canfield W M
CORPORATE SOURCE: W. K. Warren Medical Research Institute and the Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104, USA.. Bill-canfield@uokhsc.edu
SOURCE: Journal of biological chemistry, **(1996 Dec 6)** 271 (49) 31437-45.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970109

AB UDP-N-acetylglucosamine:lysosomal-enzyme **N-acetylglucosamine-1-phosphotransferase** (GlcNAc-phosphotransferase) catalyzes the initial step in the synthesis of the mannose 6-phosphate determinant required for efficient intracellular targeting of newly synthesized lysosomal hydrolases to the lysosome. The enzyme was partially **purified** approximately 30,000-fold by chromatography of solubilized membrane proteins from lactating bovine mammary glands on DEAE-Sepharose, reactive green 19-agarose, and Superose 6. The partially **purified** enzyme was used to generate a panel of murine monoclonal antibodies. The anti-GlcNAc-phosphotransferase monoclonal antibody PT18 was coupled to a solid support and used to immunopurify the enzyme approximately 480,000-fold to apparent homogeneity with an overall yield of 29%. The **purified** enzyme has a specific activity of 10-12 micromol of GlcNAc phosphate transferred per h/mg using 100 mM alpha-methylmannoside as acceptor. The subunit structure of the enzyme was determined using a combination of analytical gel filtration chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and amino-terminal sequencing. The data indicate that bovine GlcNAc-phosphotransferase is a 540,000-Da complex composed of disulfide-linked homodimers of 166,000- and 51,000-Da subunits and two identical, noncovalently associated 56,000-Da subunits.

L4 ANSWER 5 OF 25 MEDLINE on STN
ACCESSION NUMBER: 97094922 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8940156
TITLE: Bovine UDP-N-acetylglucosamine:lysosomal-enzyme **N-acetylglucosamine-1-phosphotransferase**. II. Enzymatic characterization and identification of the catalytic subunit.
AUTHOR: Bao M; Elmendorf B J; Booth J L; Drake R R; Canfield W M
CORPORATE SOURCE: W. K. Warren Medical Research Institute and the Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104, USA.. Bill-canfield@uokhsc.edu
SOURCE: Journal of biological chemistry, **(1996 Dec 6)** 271 (49) 31446-51.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970109

AB The kinetic properties of UDP-N-acetylglucosamine:lysosomal-enzyme **N-acetylglucosamine-1-phosphotransferase** (GlcNAc-phosphotransferase) **purified** to homogeneity from lactating bovine mammary gland have been investigated. GlcNAc-phosphotransferase transferred GlcNAc 1-phosphate from UDP-GlcNAc to the synthetic acceptor alpha-methylmannoside, generating GlcNAc-1-phospho-6-mannose alpha-methyl, the structure of which was confirmed by mass spectroscopy. GlcNAc-phosphotransferase was active between pH 5.7 and 9.3, with optimal activity between pH 6.6 and 7.5. Activity was strictly dependent on Mg2+ or Mn2+. The Km for Mn2+ was 185 microM. The Km for UDP-GlcNAc was 30 microM, and that for alpha-methylmannoside was 63 mM. The enzyme was competitively inhibited by UDP-Glc, with a Ki of 733 microM. The 166-kDa subunit was identified

as the catalytic subunit by photoaffinity labeling with azido-[beta-32P]UDP-Glc. **Purified** GlcNAc-phosphotransferase utilizes the lysosomal enzyme uteroferrin approximately 163-fold more effectively than the non-lysosomal glycoprotein ribonuclease B. Antibodies to GlcNAc-phosphotransferase blocked the transfer to cathepsin D, but not to alpha-methylmannoside, suggesting that protein-protein interactions are required for the efficient utilization of glycoprotein acceptors. These results indicate that the **purified** bovine GlcNAc-phosphotransferase retains the specificity for lysosomal enzymes as acceptors previously observed with crude preparations.

L4 ANSWER 6 OF 25 MEDLINE on STN
ACCESSION NUMBER: 92283889 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1317874
TITLE: Characterization of UDP-N-acetylglucosamine:glycoprotein **N-acetylglucosamine-1-phosphotransferase** from Acanthamoeba castellanii.
AUTHOR: Ketcham C M; Kornfeld S
CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: CA 08759 (NCI)
SOURCE: Journal of biological chemistry, **(1992 Jun 5)** 267 (16) 11654-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920717
Last Updated on STN: 19970203
Entered Medline: 19920706

AB The kinetic properties of UDP-N-acetylglucosamine:glycoprotein **N-acetylglucosamine-1-phosphotransferase** (GlcNAc-phosphotransferase) partially **purified** from the soil amoeba Acanthamoeba castellanii have been studied. The transferase phosphorylated the lysosomal enzymes uteroferrin and cathepsin D 3-90-fold better than nonlysosomal glycoproteins and 16-83-fold better than a Man9GlcNAc oligosaccharide. Deglycosylated uteroferrin was a potent competitive inhibitor of the phosphorylation of intact uteroferrin (Ki of 48 microM) but did not inhibit the phosphorylation of RNase B or the simple sugar alpha-methylmannoside. Deglycosylated RNase (RNase A) did not inhibit the phosphorylation of RNase B or uteroferrin. These results indicate that **purified** amoeba GlcNAc-phosphotransferase recognizes a protein domain present on lysosomal enzymes but absent in most nonlysosomal glycoproteins. The transferase also exhibited a marked preference for oligosaccharides containing mannose alpha 1,2-mannose sequences, but this cannot account for the high affinity binding to lysosomal enzymes. A. castellanii extracts do not contain detectable levels of N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase, the second enzyme in the biosynthetic pathway for the mannose 6-phosphate recognition marker. We conclude that A. castellanii does not utilize the phosphomannosyl sorting pathway despite expression of very high levels of GlcNAc-phosphotransferase.

L4 ANSWER 7 OF 25 MEDLINE on STN
ACCESSION NUMBER: 92298029 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1318762
TITLE: **Purification** and characterization of human lymphoblast **N-acetylglucosamine-1-phosphotransferase.**
AUTHOR: Zhao K W; Yeh R; Miller A L
CORPORATE SOURCE: Department of Neurosciences, University of California, San Diego School of Medicine, La Jolla 92093.
CONTRACT NUMBER: NS12138 (NINDS)

SOURCE: Glycobiology, **(1992 Apr)** 2 (2) 119-25.
Journal code: 9104124. ISSN: 0959-6658.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920731
Last Updated on STN: 19920731
Entered Medline: 19920720

AB **N-Acetylglucosamine-1-phosphotransferase** (GlcNAcPTase) was solubilized with 2% Tergitol NP-10 from cultured human lymphoblast cells and **purified** 3840-fold with 14% recovery using lentil lectin-Sepharose 4B, DEAE-Sephacel and Sephacryl S-400 chromatographies. The partially **purified** enzyme requires the non-ionic detergent Tergitol NP-10 and a divalent cation, Mn2+ or Mg2+, for its activity and exhibits an optimal pH at 7.2-7.5 in Tris-maleate buffer. Kinetic studies demonstrated an apparent Km of 24 microM for the donor UDP-N-acetylglucosamine and of 117 mM for the artificial acceptor alpha-methylmannoside. The GlcNAcPTase is inhibited by UDP and UDP-glucose, and by negatively charged phospholipids including phosphatidylserine, phosphatidylglycerol and phosphatidic acid. The apparent mol. wt of the human lymphoblast GlcNAcPTase is approximately 1000 kDa, which is analogous to that reported for the partially **purified** enzyme from rat liver (Waheed et al., 1982).

L4 ANSWER 8 OF 25 MEDLINE on STN
ACCESSION NUMBER: 92283888 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1317873
TITLE: **Purification** of UDP-N-acetylglucosamine:glycoprotein **N-acetylglucosamine-1-phosphotransferase** from Acanthamoeba castellanii and identification of a subunit of the enzyme.
AUTHOR: Ketcham C M; Kornfeld S
CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: CA 08759 (NCI)
SOURCE: Journal of biological chemistry, **(1992 Jun 5)** 267 (16) 11645-53.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920717
Last Updated on STN: 19970203
Entered Medline: 19920706

AB UDP-N-acetylglucosamine:glycoprotein **N-acetylglucosamine-1-phosphotransferase** (GlcNAc-phosphotransferase) from the soil amoeba Acanthamoeba castellanii has been **purified** over 100,000-fold by means of wheat germ agglutinin-Sepharose affinity chromatography, DEAE-cellulose chromatography, concanavalin A-Sepharose affinity chromatography, orange A-agarose dye chromatography, and gel filtration on Superose 6. The most **purified** enzyme has an estimated specific activity of at least 5 mumol of GlcNAc-phosphate transferred/min/mg of protein using alpha-methylmannoside as acceptor. The molecular weight of the native enzyme is approximately 250,000, as determined by gel filtration and glycerol gradients in H2O and D2O. A protein with an apparent M(r) of 97,000 in small scale preparations and its putative proteolytic fragment of 43,000 in large scale preparations co-purifies with the enzyme activity. This protein is covalently modified with GlcNAc-[32P]phosphate when the enzyme preparation is incubated with

[beta-32P]UDP-GlcNAc in the absence of an acceptor substrate. The labeling of the 97(43)-kDa protein requires active enzyme and is completely inhibited by the addition of the acceptor substrate alpha-methylmannoside. The GlcNAc-[32P]phosphate transferred to the protein is not bound to serine, threonine, tyrosine, or mannose residues. The 97(43)-kDa protein with covalently bound GlcNAc-P does not serve as a kinetically competent enzyme-substrate intermediate. However, preincubation of GlcNAc-phosphotransferase with UDP-GlcNAc does result in a decrease in the Vmax of the enzyme in subsequent assays. Taken together, these data are consistent with the 97(43)-kDa protein being a subunit of GlcNAc-phosphotransferase.

L4 ANSWER 9 OF 25 MEDLINE on STN
ACCESSION NUMBER: 93054671 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1331081
TITLE: Lysosomal enzyme phosphorylation. I. Protein recognition determinants in both lobes of procathepsin D mediate its interaction with UDP-GlcNAc:lysosomal enzyme **N-acetylglucosamine-1-phosphotransferase**.
AUTHOR: Baranski T J; Cantor A B; Kornfeld S
CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: CA08759 (NCI)
GM-07200 (NIGMS)
SOURCE: Journal of biological chemistry, **(1992 Nov 15)** 267 (32) 23342-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19930122
Entered Medline: 19921216

AB We have investigated the nature of a protein domain that is shared among lysosomal hydrolases and is recognized by UDP-GlcNAc:lysosomal enzyme **N-acetylglucosamine-1-phosphotransferase**, the initial enzyme in the biosynthesis of mannose 6-phosphate residues. Previously, elements of this recognition domain were identified using a chimeric protein approach. The combined substitution of two regions (amino acids 188-230, particularly lysine 203, and 265-292) from the carboxyl lobe of the lysosomal hydrolase cathepsin D into the homologous positions of the related secretory protein glycopepsinogen was sufficient to confer recognition by phosphotransferase and subsequent phosphorylation of the oligosaccharides when this chimeric protein was expressed in Xenopus oocytes. (Baranski, T. J., Faust, P. L., and Kornfeld, S. (1990) Cell 63, 281-291). The current study demonstrates that when these two regions are replaced in cathepsin D by the homologous glycopepsinogen amino acids, the resultant chimeric molecule is poorly phosphorylated. However, when either of these regions is substituted individually, the chimeric molecules are well phosphorylated. The phosphorylation of these latter chimeric proteins is dependent on the presence of procathepsin D amino lobe elements. By analyzing a series of chimeric proteins that contain all eight combinations of three consecutive segments of the entire amino lobe of procathepsin D, it was found that multiple regions of the amino lobe of cathepsin D enhance phosphorylation of the chimeric proteins. These elements may be part of an extended carboxyl lobe recognition domain or comprise a second independent recognition domain.

L4 ANSWER 10 OF 25 MEDLINE on STN
ACCESSION NUMBER: 86196049 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2939074

TITLE: Glycoprotein phosphorylation in simple eucaryotic organisms. Identification of UDP-GlcNAc:glycoprotein **N-acetylglucosamine-1-phosphotransferase** activity and analysis of substrate specificity.
AUTHOR: Lang L; Couso R; Kornfeld S
CONTRACT NUMBER: 5T32GM 07200 (NIGMS)
RO1 CA08759 (NCI)
SOURCE: Journal of biological chemistry, **(1986 May 15)** 261 (14) 6320-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198606
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19980206
Entered Medline: 19860616

AB UDP-N-acetylglucosamine:glycoprotein **N-acetylglucosamine-1-phosphotransferase** activity has been identified in both Acanthamoeba castellani and Dictyostelium discoideum. Each of these activities exhibits a different in vitro specificity toward various **purified** glycoproteins. The N-acetylglucosaminyl-phosphotransferase of A. castellani is very similar to the mammalian enzyme in that it phosphorylates the lysosomal enzymes cathepsin D and uteroferrin much more efficiently than nonlysosomal glycoproteins and appears to recognize a determinant on the protein portion of these good acceptors. In contrast the D. discoideum enzyme cannot utilize cathepsin D as a good substrate and, although it phosphorylates uteroferrin efficiently, it does not recognize the protein portion of this acceptor. The oligosaccharide of uteroferrin appears to assume a different conformation than the oligosaccharides of other glycoproteins and glycopeptides, as evidenced by its enhanced sensitivity to mannosidase digestion. This conformation, presumably induced by some interaction with the underlying protein, may be responsible for the specific phosphorylation of uteroferrin by the N-acetylglucosaminylphosphotransferase of D. discoideum.

L4 ANSWER 11 OF 25 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:786620 HCAPLUS
DOCUMENT NUMBER: 130:135758
TITLE: Identification of UDP-N-acetylglucosamine-phosphotransferase-Binding Sites on the Lysosomal Proteases, Cathepsins A, B, and D
AUTHOR(S): Lukong, Kiven E.; Elsliger, Marc-Andre; Mort, John S.; Potier, Michel; Pshezhetsky, Alexey V.
CORPORATE SOURCE: Service de Genetique Medicale, Hopital Sainte-Justine, Montreal, QC, H3T 1C5, Can.
SOURCE: Biochemistry (**1999**), 38(1), 73-80
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A key step in the targeting of sol. lysosomal enzymes is their recognition and phosphorylation by a 540 kDa multisubunit enzyme, UDP-**N-acetylglucosamine-1-phosphotransferase** (phosphotransferase). The mol. mechanism of recognition is still unknown, but previous expts. suggested that the phosphotransferase-binding sites on lysosomal proteins are represented by structurally conserved surface patches of amino acids. The authors identified four such regions on nonhomologous lysosomal enzymes, cathepsins A, B, and D, which were superimposed by rotating their structures around the C.alpha. atom of the glycosylated Asn residue. The authors proposed that these regions represent putative phosphotransferase-binding sites and tested synthetic

peptides, derived from these regions on the basis of surface accessibility, for their ability to inhibit in vitro phosphorylation of **purified** cathepsins A, B, and D. Our results indicate that cathepsin A and cathepsin D have one closely related phosphotransferase recognition site represented by a structurally and topol. conserved .beta.-hairpin loop, similar to that previously identified in lysosomal .beta.-glucuronidase. The most potent inhibition of phosphorylation was demonstrated by homologous peptides derived from the regions located on cathepsin mols. opposite the oligosaccharide chains which are phosphorylated by the phosphotransferase. The authors propose that recognition and catalytic sites of the phosphotransferase are located on different subunits, therefore, providing an effective mechanism for binding and phosphorylation of lysosomal proteins of different mol. size.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 12 OF 25 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1990:346154 BIOSIS
DOCUMENT NUMBER: PREV199039041415; BR39:41415
TITLE: HUMAN LYMPHOBLAST **N ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE** PARTIAL **PURIFICATION** AND CHARACTERIZATION.
AUTHOR(S): ZHAO K [Reprint author]; YEH R; MILLER A L
CORPORATE SOURCE: UNIV OF CALIFORNIA, SAN DIEGO, LA JOLLA, CALIF 92093, USA
SOURCE: FASEB Journal, (1990) Vol. 4, No. 7, pp. A1980.
Meeting Info.: JOINT MEETING OF THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, AND THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS, NEW ORLEANS, LOUISIANA, USA, JUNE 4-7, 1990. FASEB (FED AM SOC EXP BIOL) J.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 26 Jul 1990
Last Updated on STN: 30 Aug 1990

L4 ANSWER 13 OF 25 MEDLINE on STN

ACCESSION NUMBER: 83007346 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6288715
TITLE: UDP-N-acetylglucosamine:lysosomal enzyme precursor **N-acetylglucosamine-1-phosphotransferase**. Partial **purification** and characterization of the rat liver Golgi enzyme.
AUTHOR: Waheed A; Hasilik A; von Figura K
SOURCE: Journal of biological chemistry, **(1982 Oct 25)** 257 (20) 12322-31.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198212
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19900317
Entered Medline: 19821203

L4 ANSWER 14 OF 25 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1997:95106 BIOSIS
DOCUMENT NUMBER: PREV199799394309
TITLE: **Purification** and subunit structure of bovine UDP-N-acetyl-glucosamine:lysosomal enzyme **N-acetylglucosamine-1-phosphotransferase**.

AUTHOR(S): Bao, Ming [Reprint author]; Elmendorf, B. Jean; Booth, J. Lee; Drake, Richard L.; Canfield, William M.
CORPORATE SOURCE: WK Warren Med. Res. Inst., Univ. Oklahoma HSC, Oklahoma City, OK, USA
SOURCE: Molecular Biology of the Cell, (1996) Vol. 7, No. SUPPL., pp. 137A.
Meeting Info.: Annual Meeting of the 6th International Congress on Cell Biology and the 36th American Society for Cell Biology. San Francisco, California, USA. December 7-11, 1996.
CODEN: MBCEEV. ISSN: 1059-1524.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Mar 1997
Last Updated on STN: 3 Mar 1997

L4 ANSWER 15 OF 25 MEDLINE on STN
ACCESSION NUMBER: 94124511 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8294420
TITLE: **Purification** and kinetic parameters of bovine liver N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase.
AUTHOR: Mullis K G; Huynh M; Kornfeld R H
CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: CA08759 (NCI)
T32-HL07088 (NHLBI)
SOURCE: Journal of biological chemistry, **(1994 Jan 21)** 269 (3) 1718-26.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940314
Last Updated on STN: 19970203
Entered Medline: 19940225

AB The enzyme N-acetylglucosamine phosphodiester alpha-N-acetylglucosaminidase (phosphodiester alpha-GlcNAcase) catalyzes the second step in the formation of the mannose 6-phosphate targeting signal on lysosomal enzyme oligosaccharides by removing GlcNAc residues from GlcNAc-alpha-P-mannose moieties, which are formed in the first step by UDP-N-acetyl-glucosamine:glycoprotein **N-acetylglucosamine-1-phosphotransferase** (GlcNAc-phosphotransferase). Phosphodiester alpha-GlcNAcase, a membrane-bound enzyme, has been **purified** about 3,000-fold from bovine liver to apparent homogeneity using detergent solubilization, fractionation on DEAE-cellulose, affinity chromatography on lectin-Sepharose columns, gel filtration, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The enzyme migrated as 129- and 121-kDa species on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Since both bands had the same amino-terminal sequence, the smaller species is presumed to be derived from the larger by proteolysis. Kinetic analysis of bovine phosphodiester alpha-GlcNAcase with enzymatically synthesized artificial and biological substrates indicates that phosphodiester alpha-GlcNAcase requires GlcNAc-alpha-P R for substrate and that when R contains the Man alpha 1,2Man linkage the substrate binding is most effective. Unlike GlcNAc-phosphotransferase, bovine phosphodiester alpha-GlcNAcase does not require a protein recognition determinant on lysosomal enzyme substrates.

L4 ANSWER 16 OF 25 MEDLINE on STN

ACCESSION NUMBER: 88133837 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2829817
TITLE: Properties of **N-acetylglucosamine 1-phosphotransferase** from human lymphoblasts.
AUTHOR: Little L; Alcouloumre M; Drotar A M; Herman S; Robertson R; Yeh R Y; Miller A L
CORPORATE SOURCE: Department of Neurosciences, School of Medicine, University of California, San Diego, La Jolla 92093.
CONTRACT NUMBER: NS12138 (NINDS)
SOURCE: Biochemical journal, **(1987 Nov 15)** 248 (1) 151-9.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198803
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19880309

AB Human lymphoblast and fibroblast cell lines from a patient with I-cell disease and normal individuals were characterized with respect to certain properties of UDP-N-acetylglucosamine:lysosomal enzyme precursor N-acetylglucosamine phosphotransferase. The enzyme isolated from normal lymphoblast and fibroblast cell lines expressed similar kinetic properties, substrate specificities and subcellular localizations. Coincident with the severe reduction of N-acetylglucosamine phosphotransferase activity in both I-cell fibroblast and lymphoblast cell lines, there was an increased secretion of several lysosomal enzymes compared to normal controls. Subsequent examination of N-acetyl-beta-D-hexosaminidase secreted by the I-cell lymphoblasts demonstrated a significant increase in adsorption of the I-cell enzyme to Ricinus communis agglutinin, a galactose-specific lectin. However, the I-cell lymphoblasts did not exhibit the significant decrease in intracellular lysosomal activities seen in I-cell fibroblasts. Our results suggest that lymphoblasts not only represent an excellent source for the **purification** of N-acetylglucosamine phosphotransferase, but in addition, represent a unique system for studying alternate mechanisms involved in the targeting of lysosomal enzymes.

L4 ANSWER 17 OF 25 MEDLINE on STN
ACCESSION NUMBER: 93003385 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1390929
TITLE: Components and proteolytic processing sites of arylsulfatase B from human placenta.
AUTHOR: Kobayashi T; Honke K; Jin T; Gasa S; Miyazaki T; Makita A
CORPORATE SOURCE: Biochemistry Laboratory, Hokkaido University School of Medicine, Sapporo, Japan.
SOURCE: Biochimica et biophysica acta, **(1992 Oct 20)** 1159 (3) 243-7.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19930122
Entered Medline: 19921120

AB Previous studies have shown that mature arylsulfatase B **purified** from human sources is composed of two non-identical chains with apparent molecular masses of 43 kDa and 8 kDa. Arylsulfatase B **purified** from human placenta in the present study, however, included another 7 kDa component that could be detected only by carbohydrate staining on reducing SDS-PAGE employing the Tris-Tricine system. The 43 kDa and 7 kDa

components contained a carbohydrate moiety, but the 8 kDa one did not, as demonstrated by periodic acid-Schiff staining, Con-A lectin blotting, endo-glycosidase treatment and in vitro phosphorylation by UDP-N-acetylglucosamine: lysosomal enzyme **N-acetylglucosamine 1-phosphotransferase**. The **purified** arylsulfatase B migrated as a single polypeptide of 58 kDa on non-reducing SDS-PAGE, indicating that the three chains are linked by disulfide bonds. In order to determine the origin of the components, N-terminal sequencing of the isolated polypeptides was performed. As a result, the 43, 7 and 8 kDa components were found to commence with Ala-41, Ala-424 and Asp-466, respectively. These results suggest that after removal of the signal peptide, human arylsulfatase B undergoes proteolytic processing on at least two sites during maturation.

L4 ANSWER 18 OF 25 MEDLINE on STN
ACCESSION NUMBER: 92162201 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1311180
TITLE: Characterization of the subunits and sugar moiety of human placental and leukemic beta-glucuronidase.
AUTHOR: Tanaka J; Gasa S; Sakurada K; Miyazaki T; Kasai M; Makita A
CORPORATE SOURCE: Biochemistry Laboratory, Cancer Institute, Sapporo, Japan.
SOURCE: Biological chemistry Hoppe-Seyler, **(1992 Jan)** 373 (1) 57-62.
Journal code: 8503054. ISSN: 0177-3593.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 19920417
Last Updated on STN: 19920417
Entered Medline: 19920402

AB beta-Glucuronidase **purified** from human placenta and chronic myelogenous leukemic cells was composed of three components of 18, 64 and 80 kDa, though the relative contents of the components were different between the sources. Analysis of their N-terminal amino-acid sequences showed that the 18-kDa and 64-kDa components were derived from the 80-kDa component by cleavage between Val159 and Gly160. Furthermore, the enzyme was found to be glycosylated at Asn173 and Asn420 with high mannose-type oligosaccharides, based on the electrophoretic mobility of the components as well as the endopeptidic peptides before and after endoglycosidase treatment. The enzyme **purified** from leukemic cells was poorly phosphorylated by **N-acetylglucosamine 1-phosphotransferase** as compared to the placental enzyme.

L4 ANSWER 19 OF 25 MEDLINE on STN
ACCESSION NUMBER: 85060466 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6094964
TITLE: UDP-N-acetylglucosamine: lysosomal enzyme **N-acetylglucosamine-1-phosphotransferase**.
AUTHOR: Reitman M L; Lang L; Kornfeld S
SOURCE: Methods in enzymology, **(1984)** 107 163-72.
Journal code: 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198412
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19841226

L4 ANSWER 20 OF 25 MEDLINE on STN
ACCESSION NUMBER: 82053049 MEDLINE

DOCUMENT NUMBER: PubMed ID: 6457829
TITLE: Lysosomal enzyme targeting. N-Acetylglucosaminylphosphotransferase selectively phosphorylates native lysosomal enzymes.
AUTHOR: Reitman M L; Kornfeld S
CONTRACT NUMBER: GM 07200 (NIGMS)
R01 CA 08759 (NCI)
SOURCE: Journal of biological chemistry, **(1981 Dec 10)** 256 (23) 11977-80.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198201
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19970203
Entered Medline: 19820128

AB Lysosomal enzymes contain 6-phosphomannosyl moieties which mediate their translocation to lysosomes. This recognition marker is synthesized by the sequential action of UDP-N-acetylglucosamine:lysosomal enzyme **N-acetylglucosamine-1-phosphotransferase** and alpha-N-acetylglucosaminyl phosphodiesterase. A new assay for the N-acetylglucosaminylphosphotransferase, using alpha-methylmannoside as acceptor, is presented. Using this assay, we partially **purified** the transferase and examined its substrate specificity. The transferase exhibited a very high affinity toward lysosomal enzymes (apparent Km values of less than 20 microM) and was greater than 100-fold more efficient (Vmax/Km) when using lysosomal enzymes as acceptors as compared to nonlysosomal glycoproteins that contain high mannose oligosaccharide units. Heat denaturation of the lysosomal enzymes resulted in the loss of acceptor activity. The model compounds alpha-methylmannoside and Man5--8GlcNAc were poor acceptors. We propose that this enzyme catalyzes the initial, determining step by which synthesized acid hydrolases are distinguished from other newly synthesized glycoproteins and thus are eventually targeted to lysosomes.

L4 ANSWER 21 OF 25 MEDLINE on STN
ACCESSION NUMBER: 91004236 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2170024
TITLE: Generation of a lysosomal enzyme targeting signal in the secretory protein pepsinogen.
AUTHOR: Baranski T J; Faust P L; Kornfeld S
CORPORATE SOURCE: Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: CA 08759 (NCI)
GM-07200 (NIGMS)
SOURCE: Cell, **(1990 Oct 19)** 63 (2) 281-91.
Journal code: 0413066. ISSN: 0092-8674.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199011
ENTRY DATE: Entered STN: 19910117
Last Updated on STN: 20000303
Entered Medline: 19901119

AB Lysosomal enzymes contain a common protein determinant that is recognized by UDP-GlcNAc:lysosomal enzyme **N-acetylglucosamine-1-phosphotransferase**, the initial enzyme in the formation of mannose 6-phosphate residues. To identify this protein determinant, we constructed chimeric molecules between two aspartyl proteases: cathepsin D, a lysosomal enzyme, and pepsinogen, a secretory protein. When expressed in Xenopus oocytes, the oligosaccharides of cathepsin D were efficiently phosphorylated, whereas the oligosaccharides

of a glycosylated form of pepsinogen were not phosphorylated. The combined substitution of two noncontinuous sequences of cathepsin D (lysine 203 and amino acids 265-292) into the analogous positions of glycopepsinogen resulted in phosphorylation of the oligosaccharides of the expressed chimeric molecule. These two sequences are in direct apposition on the surface of the molecule, indicating that amino acids from different regions come together in three-dimensional space to form this recognition domain. Other regions of cathepsin D were identified that may be components of a more extensive recognition marker.

L4 ANSWER 22 OF 25 MEDLINE on STN
ACCESSION NUMBER: 1998249868 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9570829
TITLE: Synthesis of 5-azido-UDP-N-acetylhexosamine photoaffinity analogs and radiolabeled UDP-N-acetylhexosamines.
AUTHOR: Sunthankar P; Pastuszak I; Rooke A; Elbein A D; van de Rijn I; Canfield W M; Drake R R
CORPORATE SOURCE: Department of Biochemistry and Molecualr Biology, University of Arkansas for Medical Sciences, Little Rock 72205, USA.
CONTRACT NUMBER: AI37320 (NIAID)
HD31920 (NICHD)
HL-17783 (NHLBI)
SOURCE: Analytical biochemistry, **(1998 May 1)** 258 (2) 195-201.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980716
Last Updated on STN: 19980716
Entered Medline: 19980709

AB Nuleotide sugar photoaffinity analogs have proven to be useful in the identification and characterization of glycosyltransferases. A radioenzymatic synthesis of [32P]5-azido-UDP-N-acetylglucosamine has been accomplished using 5-azido-UTP, [gamma-32P]ATP, porcine N-acetylgalactosamine kinase, and Escherichia coli UDP-N-acetylglucosamine pyrophosphorylase, GlmU. This general enzymatic scheme was useful for the synthesis of [32P]5-azido-UDP-N-acetylgalactosamine and high-specific-activity [3H] or [32P]UDP-N-acetylhexosamines. A new chemical synthesis method for generating 5-azido-uridine compounds was also developed. [32P]5-Azido-UDP-N-acetylglucosamine was functionally characterized using different soluble and membrane-associated glycosyltransferases which utilize UDP-GlcNAc as a substrate. Site-specific photoincorporation was observed for partially **purified** GlmU and porcine UDP-GlcNAc pyrophosphorylase. The photoprobe also effectively photoincorporated into the alpha- and beta-subunits of **purified** bovine UDP-N-acetylglucosamine:lysosomal enzyme **N-acetylglucosamine -1-phosphotransferase**. Lastly, the photoprobe was also effective at photolabeling Streptococcus pyogenes hyaluronate synthase in membrane preparations.

L4 ANSWER 23 OF 25 MEDLINE on STN
ACCESSION NUMBER: 94003651 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8400547
TITLE: Light and heavy lysosomes: characterization of N-acetyl-beta-D-hexosaminidase isolated from normal and I-cell disease lymphoblasts.
AUTHOR: Miller A L; Norton V; Robertson R; Jenks M; Yeh R Y; Wright D
CORPORATE SOURCE: Department of Neurosciences, University of California, San Diego School of Medicine, La Jolla 92093.

CONTRACT NUMBER: NS12138 (NINDS)
SOURCE: Glycobiology, **(1993 Aug)** 3 (4) 313-8.
Journal code: 9104124. ISSN: 0959-6658.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 20000303
Entered Medline: 19931118

AB We previously reported that I-cell disease lymphoblasts maintain normal or near-normal intracellular levels of lysosomal enzymes, even though **N-acetylglucosamine-1-phosphotransferase** activity is severely depressed or absent (Little et al., Biochem. J., 248, 151-159, 1987). The present study, employing subcellular fractionation on colloidal silica gradients, indicates that both light and heavy lysosomes isolated from I-cell disease and pseudo-Hurler polydystrophy lymphoblasts possess normal specific activity levels of N-acetyl-beta-D-hexosaminidase, alpha-D-mannosidase and beta-D-glucuronidase. These current findings are in contrast to those of cultured fibroblasts from the same patients, where decreased intralysosomal enzyme activities are found. Column chromatography on Ricinus communis revealed that N-acetyl-beta-D-hexosaminidase in both heavy and light I-cell disease lysosomal fractions from lymphoblasts possesses an increased number of accessible galactose residues (30-50%) as compared to the enzyme from the corresponding normal controls. Endo-beta-N-acetylglucosaminidase H treatment of N-acetyl-beta-D-hexosaminidase from the I-cell lysosomal fractions suggests that the majority of newly synthesized high-mannose-type oligosaccharide chains are modified to complex-type carbohydrates prior to being transported to lysosomes. This result from lymphoblasts differs from previous findings with fibroblasts, where N-acetyl-beta-D-hexosaminidase from I-cell disease and pseudo-Hurler polydystrophy lysosomes exhibited properties associated with predominantly high-mannose-type oligosaccharide chains.(ABSTRACT TRUNCATED AT 250 WORDS)

L4 ANSWER 24 OF 25 MEDLINE on STN
ACCESSION NUMBER: 93054672 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1331082
TITLE: Lysosomal enzyme phosphorylation. II. Protein recognition determinants in either lobe of procathepsin D are sufficient for phosphorylation of both the amino and carboxyl lobe oligosaccharides.
AUTHOR: Cantor A B; Baranski T J; Kornfeld S
CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: CA08759 (NCI)
GM-07200 (NIGMS)
SOURCE: Journal of biological chemistry, **(1992 Nov 15)** 267 (32) 23349-56.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19990129
Entered Medline: 19921216

AB Cathepsin D is a bilobed lysosomal aspartyl protease that contains one Asn-linked oligosaccharide/lobe. Each lobe also contains protein determinants that serve as recognition domains for binding of UDP-GlcNAc:lysosomal enzyme **N-acetylglucosamine-1-phosphotransferase**, the first enzyme in the

biosynthesis of the mannose 6-phosphate residues on lysosomal enzymes. In this study we examined whether the location of the protein recognition domain influences the relative phosphorylation of the amino and carboxyl lobe oligosaccharides. To do this, chimeric proteins containing either amino or carboxyl lobe sequences of cathepsin D substituted into a glycosylated form of the homologous secretory protein pepsinogen were expressed in Xenopus oocytes. The amino and carboxyl lobe oligosaccharides were then isolated from the various chimeric proteins and independently analyzed for their mannose 6-phosphate content. This analysis has shown that a phosphotransferase recognition domain located on either lobe of a cathepsin D/glycopepsinogen chimeric molecule is sufficient to allow phosphorylation of oligosaccharides on both lobes. However, phosphorylation of the oligosaccharide on the lobe containing the recognition domain is favored. We also found that the majority of the carboxyl lobe oligosaccharides of cathepsin D acquire two phosphates, whereas the amino lobe oligosaccharides only acquire one phosphate.

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ACCESSION NUMBER: 1995:360321 BIOSIS
DOCUMENT NUMBER: PREV199598374621
TITLE: Lysine-based Structure in the Proregion of Procathepsin L Is the Recognition Site for Mannose Phosphorylation.
AUTHOR(S): Cuozzo, John W.; Tao, Kai; Wu, Qi-Long; Young, Wen; Sahagian, G. Gary [Reprint author]
CORPORATE SOURCE: Dep. Physiol., Tufts Univ. Sch. Med., 136 Harrison Ave., Boston, MA 02111, USA
SOURCE: Journal of Biological Chemistry, (1995) Vol. 270, No. 26, pp. 15611-15619.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Aug 1995
Last Updated on STN: 30 Aug 1995

AB The recognition of lysosomal enzymes by UDP-GlcNAc: lysosomal-enzyme GlcNAc-1-phosphotransferase (phosphotransferase) is mediated by a protein structure on lysosomal enzymes. It has been previously demonstrated that lysine residues are required for phosphorylation of procathepsin L and are a common feature of the site on many lysosomal proteins. In this work, the procathepsin L recognition structure was further defined by identification of the region of the protein containing the structure and the critical lysine residues involved. Removal of the cathepsin L propeptide by low pH-induced autocatalytic processing abolished phosphorylation. The addition of either the **purified** propeptide or a glutathione S-transferase-propeptide fusion protein to the processed protein restored phosphorylation. Mutagenesis of individual lysine residues demonstrated that two propeptide lysine residues (Lys-54 and Lys-99) were required for efficient phosphorylation of procathepsin L. By comparison of the phosphorylation rates of procathepsin L, lysine-modified procathepsin L, and the procathepsin L oligosaccharide, lysine residues were shown to account for most, if not all, of the protein-dependent interaction. On this basis, it is concluded that the proregion lysine residues are the major elements of the procathepsin L recognition site. In addition, lysine residues in cathepsin D were shown to be as important for phosphorylation as those in procathepsin L, supporting a general model of the recognition site as a specific three-dimensional arrangement of lysine residues exposed on the surface of lysosomal proteins.

=> d his

(FILE 'HOME' ENTERED AT 11:30:02 ON 10 DEC 2004)

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, EMBASE' ENTERED AT 11:30:24 ON 10 DEC 2004

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L1          282 S N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE AND 1975-1999/PY
L2          118 DUP REM L1 (164 DUPLICATES REMOVED)
L3           25 S L2 AND (PURIFIED OR PURIFICATION OR ISOLATION)
L4           25 FOCUS L3 1-

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
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